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A SYNDROME OF GONADAL DYSGENESIS: A VARIETY OF OVARIAN AGENESIS WITH ANDROGENIC MANIFESTATIONS*

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THE condition now generally known as ovarian agenesis has been thoroughly defined by Varney, Kenyon and Koch, 1942 (1), Albright, Smith and Fraser, 1942 (2), Wilkins and Fleischmann, 1944 (3) and Lisser, Curtis, Escamilla and Goldberg, 1947 (4). The syndrome consists of: a) primary amenorrhea, b) lack of development of primary and secondary sexual characteristics, c) increased urinary gonadotropin titer, often with d) short stature (usually 53–57 inches) and e) associated congenital anomalies, of which webbed neck, cubitus valgus, and coarctation of the aorta are the most frequent (Turner, 1938 (5)). The genital maldevelopment consists of: a) atrophic external genitalia, *including the clitoris*, b) rudimentary uterus, c) somewhat small, but complete, fallopian tubes and d) ovarian anlagen represented by elongated streak-like structures, white and glistening, on the posterior surface of each broad ligament, extending parallel to the

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tubes, from the uterine cornu to the fimbriated end. Histologically, these ovarian anlagen contain only rudimentary stroma and no primordial follicles or germinal epithelium.

The purpose of this report is to present 2 cases which fulfill these criteria but which also present virilization and, in the ovarian anlagen, epithelioid cells which appear to be secretory.

CASE REPORTS

Case 1. I. P. (University of California Hospital No. 220733). This patient was an unmarried white woman, $26\frac{6}{12}$ years old, referred to us on January 4, 1954 by Dr. A. L. Abbett of San Leandro, California, because of primary amenorrhea, lack of breast development, and short stature. Her shortness was unusual for her family. Her father was over 6 feet tall, and 5 brothers were of average height, including 1 who was 6 feet tall. Her mother was 5 feet 2 inches in height, and a maternal aunt was said to be almost 6 feet tall. Her 2 sisters, aged 8 and 14, were said to be normal, and the older had menstruated.

The patient first noted growth of pubic hair when she was approximately 16 years old, but had never had any axillary hair. She was always the shortest in her class, never had a spurt of growth, but continued to grow until she was 21 years old. She had not had hot flushes or any undue feeling of warmth. There were no symptoms of hypothyroidism or of mental sluggishness.

Upon examination, she was found to be $55\frac{7}{8}$ inches tall, with a span of $58\frac{1}{2}$ inches. Symphysis to crown measured $26\frac{3}{8}$ inches and symphysis to soles, $29\frac{1}{4}$ inches. Her weight was 70 pounds. As seen in Figure 1, she was a short girl who had many moles and soft fibromata. Her carrying angle measured approximately 30°. Axillary hair was absent, pubic hair was sparse (Fig. 1D), but her face and extremities bore an excessive growth of fine hair. There was no acne. The voice was feminine. She had no palpable breast tissue and the nipples were widely separated (Fig. 1A). The neck was not webbed, and the thyroid gland was normally palpable. Her pulse rate was 76 per minute, the blood pressure was 120/80 mm. of mercury in either arm, and femoral and pedal pulses were readily palpable. No anomalies of the heart could be detected by physical examination. The hands were of normal size and shape and therefore appeared disproportionately large for this small woman. The teeth likewise were full size and the upper third molars had erupted.

Examination of the genitalia showed the labia majora to be well developed. *The clitoris was definitely hypertrophied* (Fig. 1C). The labia minora were poorly developed and on initial inspection were adherent, except for an orifice 5 mm. in diameter opposite the urethral meatus. Simple manipulation easily separated the conglutination and revealed a vaginal introitus approximately 7 mm. in diameter. On probing of the vagina, its depth was estimated to be 6–7 cm. The interior of the vagina was not visualized. Upon rectal examination, a cylindrical mass, estimated at 0.5×2 cm., was palpated in the usual uterine location. Adnexae could not be felt.

On the basis of the history and physical examination, the diagnosis of ovarian agenesis was made, but the presence of hirsutism and clitoridal hypertrophy was perplexing. Laboratory examinations gave the following results: hemoglobin 15.5 Gm. per cent, hematocrit 47 per cent, white blood cell count 4,900 per cu. mm., polymorphonuclear cells 43 per cent, and lymphocytes 57 per cent. Blood Kolmer and Kahn reactions were negative. Urinalysis did not disclose any abnormalities. The skeletal age was 13 years

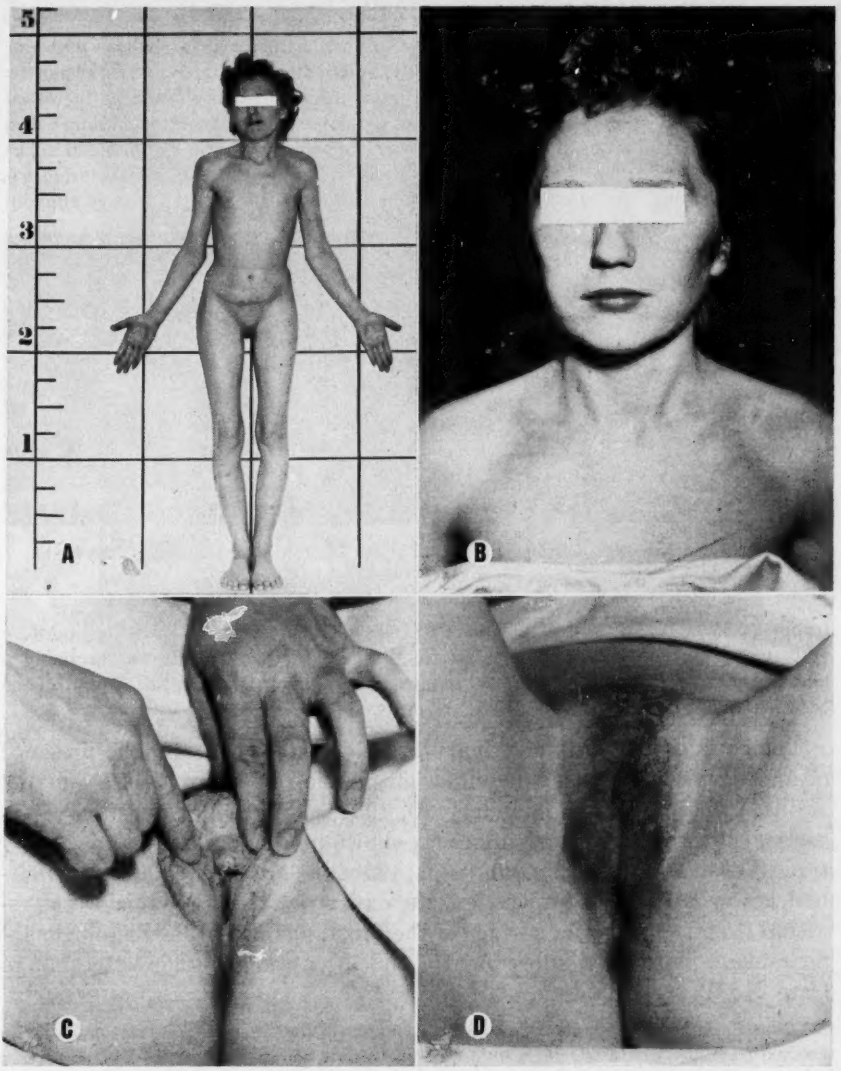


FIG. 1. **A.** Case No. 1. Note short stature, absence of mammary development, widely spaced nipples and cubitus valgus. **B.** Close-up of face: there is a fine growth of blonde hair on cheeks and upper lip. (This is more evident clinically; it photographs poorly.) Note also nevi on face, neck, and chest. **C.** Close-up of genitalia: note enlarged clitoris and poorly developed labia minora. **D.** Pubic hair, restricted to vulva and lower mons.

and 3 months, according to the standards of Greulich and Pyle (6). On roentgen pelvimetry certain masculine characteristics were manifest, namely: 1) a shallow posterior half of the pelvic inlet, 2) a narrow sacro-sciatic notch, and 3) a high, and rather narrow pubic arch (Fig. 2). X-ray films of the long bones and the pelvis showed no increased radiolucency. X-ray films of the chest showed a normal cardiac silhouette and lung fields. Excisional biopsy of a mole was performed; histologically, it was a junctional nevus (Dr. F. A. Torrey). The vaginal smear showed atrophic cells. The 17-ketosteroid excretion was 5.7 mg. per twenty-four hours. The urinary excretion of pituitary gonado-

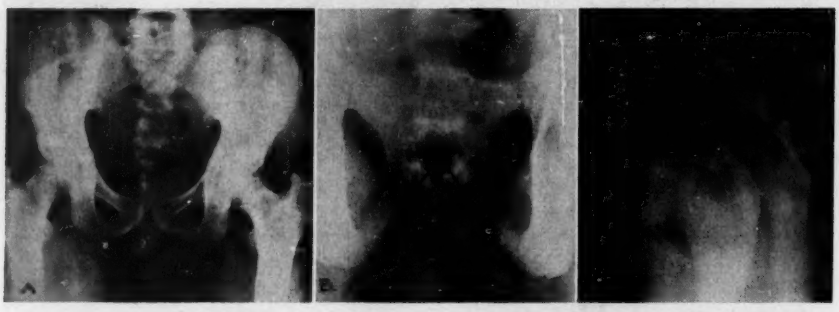


FIG. 2. Case No. 1. Roentgen pelvimetry: note android characteristics (see text), normal radiolucency, and retarded skeletal age.

tropins (method of Klinefelter, Albright and Griswold (7)) was at first reported positive at 5 mouse uterine units, and negative at 40 mouse units; but this was probably a laboratory error, for excretion was subsequently found to be positive at 160 m.u. on one occasion and borderline positive at 80 m.u. on two other occasions.

The equivocal reports of urinary gonadotropin levels and the presence of androgenic phenomena required clarification of the patient's gonadal status. Consequently, on January 20, 1954, exploratory laparotomy was carried out. The operative findings are shown in Figure 3; they were grossly typical of ovarian agenesis. On palpation of the adrenal regions no abnormalities were found. At operation, the entire right ovarian anlage was resected from the posterior surface of the broad ligament. The histologic appearance is illustrated subsequently and discussed together with that in Case No. 2.

Case 2. I. H. (University of California Hospital No. 221017). This patient was an unmarried Negro girl, $17\frac{2}{12}$ years old, referred to us on January 11, 1954 by Dr. George Degnan of Martinez, California, because of primary amenorrhea, lack of breast development, short stature, and hypertension. Nothing could be ascertained of her familial history or early development, as she was separated from her mother when only 5 months old. She was always the shortest in her class; she reported growth of approximately 1 inch in the previous year. There were no symptoms of mental retardation. She had experienced orgasm during sexual intercourse, and she described typical hot flushes from early childhood.

On physical examination her height was $56\frac{1}{2}$ inches, with a span of $62\frac{1}{2}$ inches.

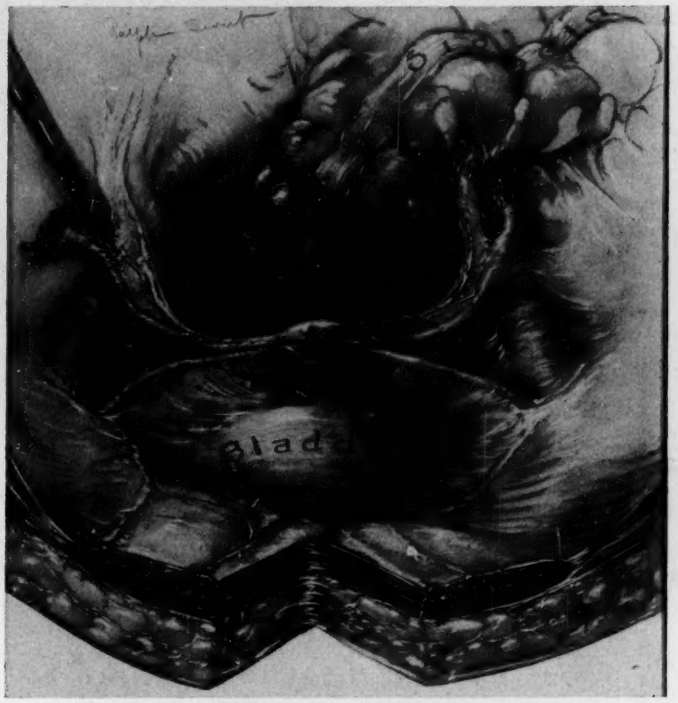


FIG. 3. Case No. 1. Gross operative findings, typical of ovarian agenesis.

Symphysis to crown measured 26¾ inches, and symphysis to soles, 29¾ inches. Her weight was 103 pounds. As seen in Figure 4A, she was a short girl who had several scattered moles on her trunk. There was no acne. The voice was feminine. Her carrying angle measured approximately 25°. Axillary hair was sparse, pubic hair was slightly less than normal (Fig. 4B), and there was a definite mustache, as well as lanugo on the arms and legs (Fig. 4A). She had no palpable breast tissue and the nipples were widely spaced. The neck was short but not webbed. The thyroid gland was normally palpable. The pulse rate was 108 per minute, and the blood pressure was 155/90–100 in the arms and 170–190/100–115 mm. of mercury in the legs. Femoral and pedal pulses were readily palpable. Examination of the heart by Dr. John J. Sampson disclosed normal size and the presence of a split and slightly accentuated second pulmonic sound, functional murmurs at the left basal and the apical areas, but no evidence of organic heart disease. The hands were disproportionately large, but graceful, with long tapering fingers.

Examination of the genitalia showed well developed labia majora. *The clitoris was definitely hypertrophied, measuring approximately 7 mm. × 25 mm.* (Fig. 4B). The labia minora were poorly developed but the vaginal introitus admitted one finger with ease and the vaginal canal was approximately 11 cm. deep. The cervix was easily visualized, measuring approximately 13 mm. in diameter, and had a normal-appearing external os, from which issued a considerable amount of clear mucus. A moderate amount of desquamated material was present in the vagina. On rectal examination, a cylindrical

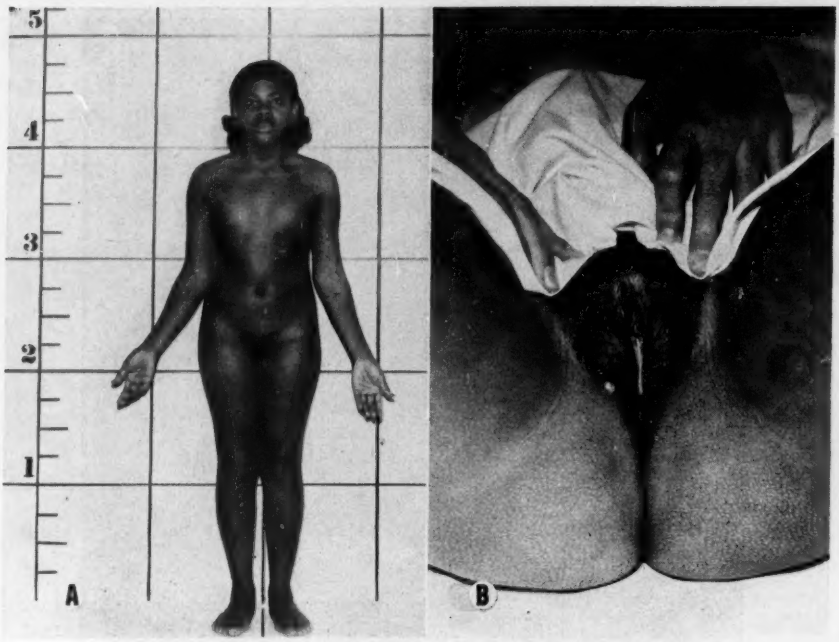


FIG. 4. **A.** Case No. 2. Note short stature, absence of breasts, and the growth of hair on the upper lip. **B.** Close-up of genitalia: note enlarged clitoris.

structure approximately 1×3 cm. was palpated in the usual location of the uterus. No adnexal structures could be palpated on vaginal or rectal examination.

Again, a diagnosis of ovarian agenesis was made but, as in Case No. 1, the androgenic phenomena required explanation. Laboratory studies showed: hemoglobin 12.5 Gm. per cent, hematocrit 42 per cent, white blood cell count 11,400 per cu. mm., polymorphonuclear leukocytes 75 per cent, lymphocytes 21 per cent, and monocytes 4 per cent.

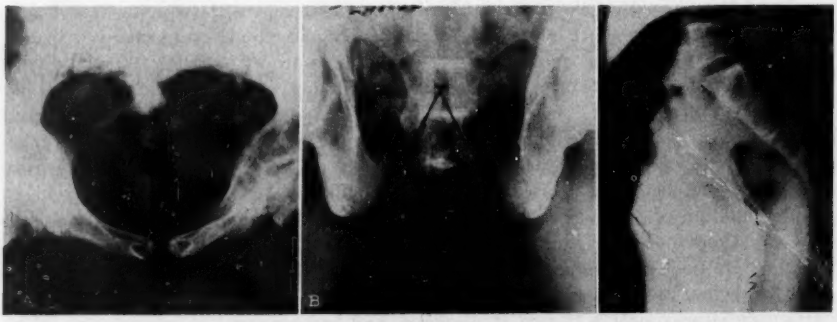


FIG. 5. Case No. 2. Roentgen pelvimetry: note android characteristics (see text), heavy bone structure, and retarded skeletal age.

FIG. 6. Case No. 2. Operative findings: the ovarian anlagen are the cylindrical structures medial to the tubes.

Blood Kolmer and Kahn reactions were negative. Urinalysis did not disclose any abnormalities. The skeletal age was 13 years and 3 months, according to the standards of Greulich and Pyle (6). On roentgen pelvimetry certain masculine characteristics were evident, similar to those of Case No. 1; in addition, there was a somewhat heavy bony structure (Fig. 5A, B and C) (8). In x-ray films of the long bones and pelvis there was no increased radiolucency. X-ray films of the chest showed a normal cardiac silhouette and lung fields; there was no scalloping of the ribs. The vaginal smear was difficult to evaluate because of the presence of a mild infection suggestive of trichomoniasis, although the organisms were not identified. Some of the vaginal cells showed maturation through the pavement and precornified stages, with apparent arrest at the latter level. This degree of cornification might be explained by the infection. The 17-ketosteroid excretion was 7.2 mg. and 5.1 mg. per twenty-four hours. The urinary content of pituitary gonadotropins was positive at 80 m.u. and negative at 160 m.u.

Because of her hypertension, the patient was subjected to a Regitine test. After intravenous administration of 5 mg. of Regitine, her blood pressure remained essentially unchanged.

Exploratory laparotomy was carried out on February 18, 1954. The operative findings are portrayed in Figure 6. The essential features of ovarian agenesis were present, except that the ovarian anlagen were definite cylindrical structures, approximately 2–3 mm. in diameter. One third of each of these structures was resected for histologic examination. On palpation of the adrenal areas no abnormalities were discovered.

Histologic findings

In both cases, the histologic appearance was similar (Figures 7 A and B; and 8 A and B). A connective-tissue stroma was present, which resembled ovarian stroma. No primordial follicles or germinal epithelium were seen. Scattered nests of epithelioid cells were noted. These cells were large and polyhedral: they contained a rather clear, lightly pink-staining cytoplasm and frequent large brightly eosinophilic granules, which were suggestive of lipoid secretion. The nuclei were small and eccentrically placed. No doubly refracting bars were seen.

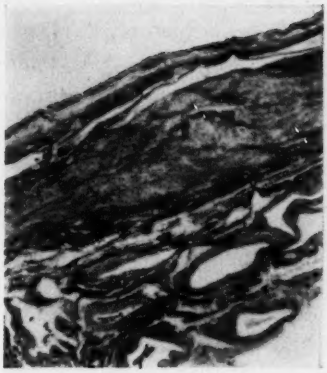
These cells did not resemble adrenal-rest cells but were reminiscent of cells of hilar or thecal origin.

TREATMENT

Patient No. 2 was given Premarin® on a schedule of 1.25 mg. daily for twenty-one days, followed by a seven-day period of no therapy. Patient No. 1 was first given cyclic treatment in the same dose for thirty-nine days and a rest period of eleven days, with the expectation of adjustment to the same cycle as in Patient No. 2. At the end of the first cycle Patient No. 1 experienced withdrawal bleeding. Patient No. 2 did not experience withdrawal bleeding until the dose of Premarin® was increased to 2.5 mg. daily for twenty-three days each month. In both patients there was development of breast tissue as well as appreciable uterine growth. Patient No. 2 reported cessation of hot flushes. Vaginal smears from both patients showed cornification of the vaginal mucosal epithelium.

DISCUSSION

Robert Meyer in 1925 (9) and Baer in 1927 (10) each reported an autopsy case of ovarian agenesis plus masculinizing features. A third case was added by Gertraude Pich in 1937 (11). She noted in her review of the cases of ovarian agenesis reported up to 1937, that the 3 patients who had masculinizing features also had groups of epithelioid cells in the ovarian anlagen resembling interstitial or hilar cells ("Zwischenzellen [sog. Hiluszellen]"). Epithelioid cells were also seen in another case (R. Meyer (12)), but there was no masculinization. A fifth patient (Rössle and Wallart (13)) also showed similar cells, but no masculinization. On the other hand, in the 4 cases of ovarian agenesis without masculinizing features col-



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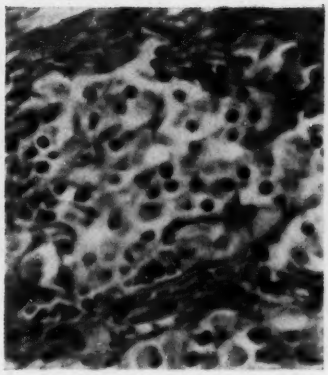

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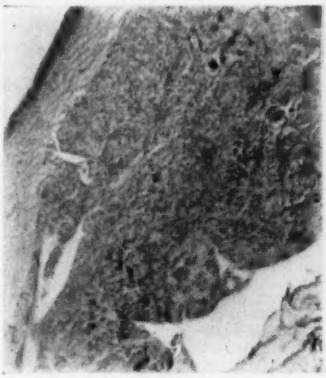

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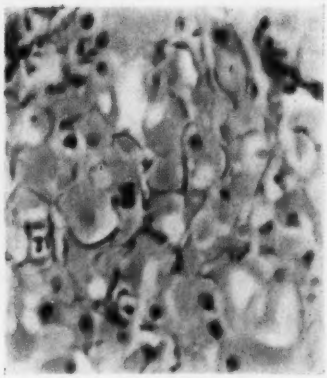

8B

FIG 7. **A**. Case No. 1. Photomicrograph of ovarian anlage ×50. Masson's stain. (See text for description.) **B.** Higher magnification (×400), to show epithelioid cells (see text).

FIG. 8. **A.** Case No. 2. Photomicrograph of ovarian anlage ×50. **B.** Higher magnification (×400), to show larger epithelioid cells

lected by Pich, there was no mention of the presence of such epithelioid cells in the ovarian anlagen (Pich, Cases 1 and 3; Olivet (14); and Schürmann (15)). Pich's patient, interestingly, also had a rudimentary prostate. The many cases of ovarian agenesis reported since Pich's classic paper have shown no masculinizing phenomena except for clitoridal hypertrophy in 2 of the 7 patients with rudimentary ovaries reported by del Castillo *et al.* (16). In 1 of these 2 subjects, biopsy material from "the border of a section of the tube" contained "a small conglomerate of cells . . . , possibly an inclusion of aberrant cells of the adrenal cortex." This patient also had increased 24-hour 17-ketosteroid excretion (24.1, 28.0, 16.3, and 16.9 mg.). Both of these patients with clitoridal hypertrophy, however, did show generalized osteoporosis and were not reported as being hirsute. The histologic description in both instances does not permit a decision as to whether these cases entirely fit the criteria for the new syndrome.

In an attempt to determine whether the secretory cells of the ovarian anlagen behave like interstitial cells, we measured the effect of chorionic gonadotropin upon the 17-ketosteroid excretion of our 2 patients. In Case No. 1, the experiment was performed postoperatively, after removal of one of the two ovarian anlagen. In Case No. 2, chorionic gonadotropin was administered up to four days prior to laparotomy. The results are tabulated in Table 1. It will be noted that the administration of 25,000 I.U. of chorionic

TABLE 1. EFFECT OF APL ADMINISTRATION ON 17-KETOSTEROID EXCRETION
(APL dosage = 5000 units daily for five days)

	17-KS before APL (mg./24 hrs.)	17-KS after APL (mg./24 hrs.)	
		4th day	5th day
Case 1	5.7	8.4	6.5
Case 2	7.2 5.1	0.8	5.7

gonadotropin intramuscularly over a period of five days failed to cause any significant increase in the 17-ketosteroid output. At least in this respect, the secretory cells do not behave like interstitial cells. Histologically, they somewhat resemble interstitial cells, but lack Reinke's bars. Robert Meyer considered the cells neither those of ovarian follicles nor of seminal tubules. He thought them most nearly like granulosa cells. It is of interest that the cells in our Case 2 (Fig. 8 B) were larger than those in Case No. 1 (Fig. 7 B) and appeared somewhat more active. Possibly, the treatment with chorionic gonadotropin prior to laparotomy in Case No. 2 played a role in this difference.

Nature and source of the androgen

It is of interest that 1 of our 2 patients and 2 of the previously reported cases with masculinization (those of Pich and Baer) had no axillary hair, despite the presence of sufficient androgen to cause facial hirsutism and clitoridal hypertrophy. On the other hand, the patient of Meyer (9) and the 2 patients of del Castillo *et al.* (16) did have axillary hair. In our cases the lack of increased 17-ketosteroid excretion and failure of the 17-ketosteroid output to increase after the administration of chorionic gonadotropin indicate that the androgen elaborated is not testosterone. It is not likely that the androgenic phenomena are simply the result of a lowered estrogen/androgen ratio; for if such were the case, all patients with ovarian agenesis or the menopause would manifest these abnormalities. An adrenal origin for the excess androgen present is also unlikely because: 1) in the autopsy cases previously described the adrenal cortex was found to be normal; 2) palpation of the adrenal areas in our 2 cases disclosed no abnormalities; 3) clinically, the syndrome diverges considerably from the known types of adrenal virilism—there is no heavy musculature, thick growth of axillary hair or masculine pubic hair (except in Meyer's case of 1925), urogenital sinus, increased output of 17-ketosteroids, or accelerated skeletal age.

Clinically, the present syndrome also differs from that of true hermaphrodism, since feminine hypogonadism, short stature, the associated congenital anomalies of ovarian agenesis (cubitus valgus, hypertension, moles) and increased excretion of gonadotropins are present.

It is worthy of note that androgenic influence sufficient to produce facial hirsutism and clitoridal hypertrophy did not cause an acceleration of skeletal maturation. Indeed, these 2 patients manifested a greater degree of skeletal retardation than the average case of ovarian agenesis. This observation is in harmony with our experience with the administration of methyltestosterone (17).

Although the androgen presumably is secreted by the epithelioid cells of the ovarian anlagen, it should be noted that 2 cases have been reported which showed these cells but no masculinization (12, 13).

SUMMARY

Two cases of ovarian agenesis with mild virilization are described. In addition to the usual findings of ovarian agenesis—primary amenorrhea, lack of development of breasts and of primary and secondary sexual characteristics, delayed skeletal maturation, and increased excretion of gonadotropins—these 2 patients also had clitoridal hypertrophy, hirsutism, and android pelves. There was no osteoporosis. In both cases, biopsy of the ovarian anlagen disclosed the presence of ovarian-like stroma, absence of

primordial follicles and germinal epithelium, and the presence of epithelioid cells containing eosinophilic granules suggestive of lipoid secretion. Administration of chorionic gonadotropin failed to increase the slightly low 17-ketosteroid excretion.

This syndrome, of which 3 unequivocal cases and 2 possible cases have been collected from the literature, appears to be the result of a combination of congenital anomalies. These are: hypoestrinism from ovarian agenesis, and androgenic manifestations which we attribute to secretion of androgen from epithelioid cells in the ovarian anlagen, together with the other congenital anomalies frequently seen in patients with ovarian agenesis (short stature, cubitus valgus, hypertension, and moles).

Addendum

Since this manuscript was submitted, Drs. Albert Segaloff and William H. Sternberg have kindly reviewed the histologic sections of the rudimentary gonads of our 2 patients. They state, "The sections on Case No. 2 represent hilus cells, despite the fact that there are no Reinke crystalloids. The sections on Case No. 1 probably represent hilus cells, but we are not as well convinced, because there is not enough material on the slides to be really certain." Regarding Case No. 2 of del Castillo, de la Balze and Argonz (our reference No. 16), they state, "We were fortunate enough to obtain some slides of the ovarian remnant here described as containing aberrant adrenal tissue, and we feel that the lesion is unequivocally that of hilus cell hyperplasia." This case, then, should probably be included as a definite instance of the present syndrome, bringing the total number of recorded cases to 6.

Also, in 1954, Poloni, Hunter and Lennox (*Lancet* **2**: 120 (July 17) 1954) and Wilkins, Grumbach and Van Wyk (*J. Clin. Endocrinol. & Metab.* **14**: 1270 (Oct.) 1954) found sex chromosome counts in the skin of patients with ovarian agenesis to be of either genetic male or genetic female pattern. Of their 11 cases, 6 were read as male and 5 as female. We are indebted to Dr. Charles Miles for a sex chromosome count in the skin of our Case No. 1. This was reported to match the male pattern. In another case of ovarian agenesis without androgenic phenomena, he found a male pattern. Wilkins *et al.*, in the light of 3 other studies, suggested that "male sexual differentiation occurs only if testes are present to antagonize the inherent tendency of the fetus of placental mammals to feminize." Thus it may well be that the present syndrome describes gonadal agenesis in genetic males. It is noteworthy that Robert Meyer in 1925 (our reference No. 9) described his case as "hermaphroditismus externus asexualis."

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CHANGES IN PLASMA LEVELS OF 17-HYDROXY-CORTICOSTEROIDS DURING THE INTRAVENOUS ADMINISTRATION OF ACTH. II. RESPONSE UNDER VARIOUS CLINICAL CONDITIONS*

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IN a previous paper from our laboratory (1), a test was described for the evaluation of adrenocortical capacity, based on changes in the circulating levels of 17-hydroxycorticosteroids in blood plasma during the intravenous infusion of 25 I.U. of adrenocorticotropic hormone (ACTH) over six hours. No significant difference was found between plasma 17-hydroxycorticosteroid levels following the administration of 25 I.U. of ACTH over six hours as compared with 50 I.U. of ACTH during the same period. Furthermore, we were able to show that the administration of ACTH over six hours caused maximal stimulation of the adrenal cortex in the resting human. Continuous intravenous administration of optimal doses of ACTH—25 I.U. every six hours—did not raise significantly the levels of 17-hydroxycorticosteroids above those achieved after the six-hour infusion. It was pointed out that, for the test to be quantitatively significant, a stable adrenocortical steroid environment is required. This can be found under physiologic conditions or under pathologic conditions in which a balance between production and elimination of adrenal hormone has been established.

In this paper the test of adrenocortical capacity has been applied in various clinical conditions.

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† Baxter and Fulbright Research Fellow.

‡ Scholar in Cancer Research of the American Cancer Society. Work performed during the tenure of a Research Fellowship of the Dazian Foundation for Medical Research (1952–53).

METHODS

Adrenocorticotropic hormone was dissolved in 0.9 per cent sodium chloride or 5 per cent glucose in water and administered by intravenous infusion over six hours. Most of the ACTH tests were performed with lot number K56409 of "crude" ACTH (Armour). Other tests were made with Wilson ACTH (lot number 58792), Upjohn ACTH (lot number CB2443ZF) and corticotropin "Nyco" (produced in Norway from the pituitary of the whale). As reported elsewhere (1) no significant differences were found between different ACTH preparations in amounts of 25 I.U. given intravenously over six hours.

Plasma 17-hydroxycorticosteroids were determined by a modification of the method of Nelson and Samuels (2, 3). Each determination was carried out on 10 ml. of plasma. It should be stressed that the method determines not only 17-hydroxycorticosterone (hydrocortisone) but other chloroform-extractable 17,21-dihydroxy-20-ketosteroids, including the dihydro and tetrahydro derivatives of cortisone and 17-hydroxycorticosterone. The conjugated forms of these steroids are, however, not determined by the present method, since they are not extracted and eluted by the solvents used. Since it has been demonstrated by Bush and Sandberg (4) that the major portion of circulating free corticosteroids in the human consists of 17-hydroxycorticosterone (compound F of Kendall), and that following the administration of ACTH the preponderance of this steroid becomes even more marked, it is apparent that the method used in this paper does determine largely 17-hydroxycorticosterone.

RESULTS

In patients with clinically typical Addison's disease the intravenous administration of ACTH induced little or no rise in 17-hydroxycorticosteroid levels (Table 1). This group, however, seemed to fall into two categories, as judged by the plasma 17-hydroxycorticosteroid levels. In 3 of these determinations there were no measurable concentrations before the test, and none could be demonstrated following ACTH administration. In the other 3, (2 of which were on the same patient) 17-hydroxycorticosteroid levels were within the normal range but could not be raised significantly with the standard ACTH test.

The changes in plasma 17-hydroxycorticosteroid levels during the stand-

TABLE 1. PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS DURING THE INTRAVENOUS INFUSION OF 25 I.U. OF ACTH OVER AN EIGHT-HOUR PERIOD TO SUBJECTS WITH CHARACTERISTIC ADDISON'S DISEASE

Subject	Plasma 17-OH-CS levels (gamma/100 ml.)					
	0 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	24 hrs.
A	0	0	0	0	0	
B	0	0	0	0	0	
C	14	12	12	14	12	15
C	13	12	12	13	13	13
D	11	9	8	7	7	
F	0	0	0	0	0	

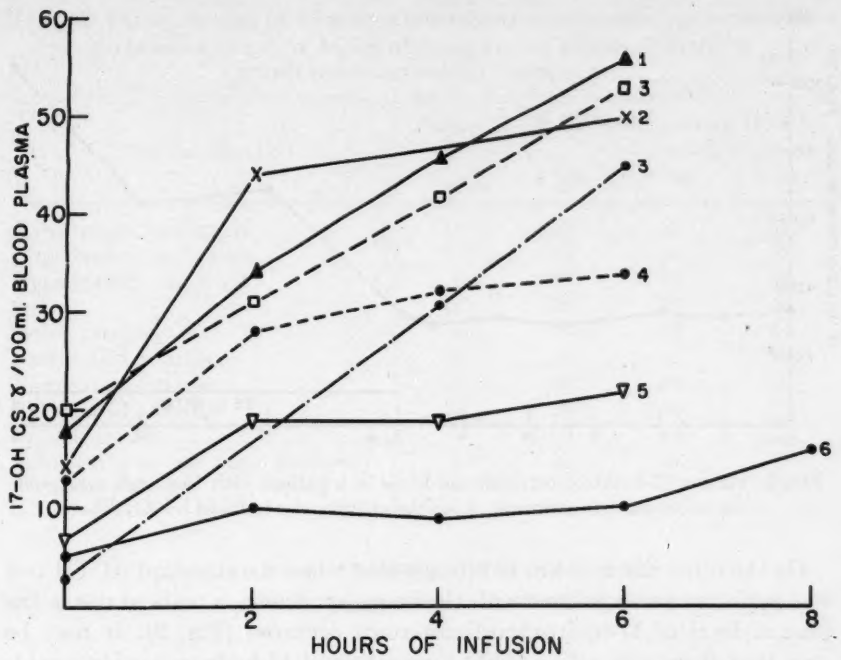

FIG. 1. Plasma 17-hydroxycorticosteroid levels during the administration of 25 I.U. of ACTH intravenously over a period of six to eight hours to 6 subjects with suspected adrenal insufficiency. Only Subjects 5 and 6 showed impaired adrenocortical capacity.

ard ACTH test on a series of subjects suspected of adrenal insufficiency are shown in Figure 1. The first 4 subjects showed responses of normal magnitude (the response in normal subjects may be seen in Figure 3) and their subsequent clinical courses did not indicate definite adrenal insufficiency. The rise in Subject 5 was at the lower limit of normal but was definite. This patient had hyponatremia and died of tuberculosis, but at autopsy the adrenals appeared normal. Subject 6 was a patient with advanced pulmonary tuberculosis, hypotension, salt craving, but no pigmentation of the Addisonian type. Although the patient showed some rise in the plasma 17-hydroxycorticosteroid level following the standard ACTH test, the small magnitude of this rise suggested that he had negligible adrenal capacity. His subsequent clinical course and especially his response to desoxycorticosterone acetate supported the conclusion. Five months after the test no demonstrable levels of 17-hydroxycorticosteroids were found in this patient's plasma. Approximately eight months after the last ACTH test he died, and on autopsy no significant amounts of adrenocortical tissue could be found.

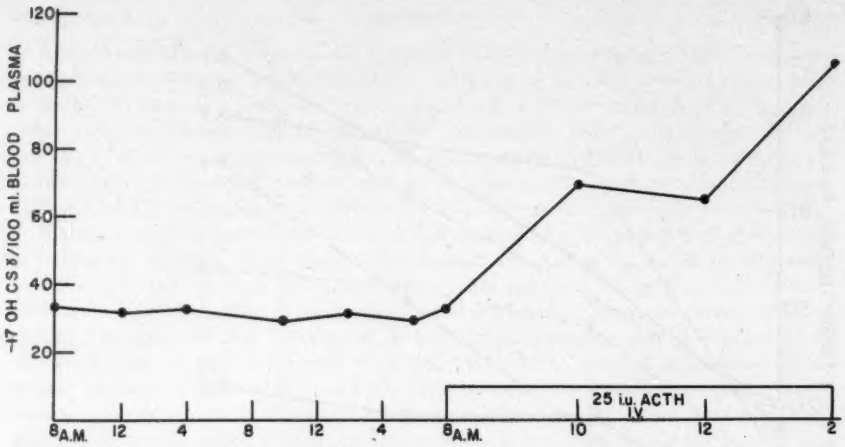

FIG. 2. Plasma 17-hydroxycorticosteroid levels in a patient with Cushing's syndrome due to an adrenal adenoma. Note the striking rise induced by ACTH.

On the other hand, it was of interest that when the standard ACTH test was performed on a patient with Cushing's syndrome, a marked rise in the plasma level of 17-hydroxycorticosteroids occurred (Fig 2). It may be seen that there was only a slight fluctuation of 17-hydroxycorticosteroids before the ACTH test, thus showing the stability of the adrenocortical environment. Under these circumstances adrenocortical capacity could be measured. At operation this patient proved to have a large adrenocortical adenoma, which was remarkable because the normal architectural pattern of the adrenal cortex could be distinguished.

Figure 3 shows the average 17-hydroxycorticosteroid response of a group of 8 diabetics, ranging in age from 17 to 62 years. The diabetes in all cases was considered to be of moderate to marked severity, as judged by the amount of insulin required and the occurrence of episodes of acidosis. The diabetes was under satisfactory clinical control in all patients at the time of the experiments. Although the slope of the mean values in the diabetics was somewhat greater when compared with the standard response to ACTH in the normal resting human, the difference was not statistically significant.

The responses of the plasma 17-hydroxycorticosteroids to the ACTH test in clinical conditions in which adrenocortical abnormalities were implicated are shown in Table 2. In general, the responses of this group were within the normal range. It should be pointed out that in none of these subjects were the control levels of plasma 17-hydroxycorticosteroids above or below the normal range of 3–22 gamma per 100 ml. (5).

TABLE 2. PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS DURING THE INTRAVENOUS INFUSION OF 25 I.U. OF ACTH OVER A SIX-HOUR PERIOD TO PATIENTS WITH VARIOUS PATHOLOGIC CONDITIONS

Type of disorder	Plasma 17-OH-CS levels (gamma/100 ml.)			
	0 hr.	2 hrs.	4 hrs.	6 hrs.
Hypertension, malignant	11	23	28	51
Hypertension, malignant	11	28	54	61
Hypertension, malignant	8	38	68	48
Sprue	18	29	38	40
Severe dermatitis	6	34	52	54
Rheumatoid arthritis	18	34	46	56
Rheumatoid arthritis	16	17	58	56
Rheumatoid arthritis	11	29	54	61
Aplastic anemia	6	29	34	35
Eosinophilia (severe, cause unknown)	4	20	25	39
Turner's syndrome	9		37	52

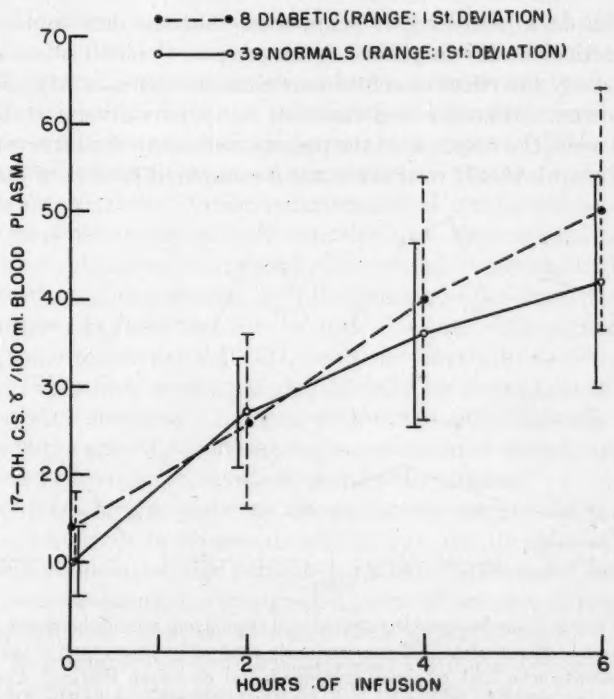

FIG. 3. Comparison of adrenocortical capacity in normal subjects and diabetic patients.

It has been shown (6) that the administration of corticosteroids over a long period of time depresses adrenocortical function and may actually cause cortical atrophy. Series of ACTH tests were carried out on 2 patients who had been receiving large oral doses of cortisone every day for a prolonged period. The test was first performed twelve hours after discontinuing the cortisone therapy and was repeated on the three following days. As seen in Figure 4, the initial levels were nil and the response to ACTH on the first test was very slight. The response to the second test was subnormal for Subject 1 and within the normal range for Subject 2. By the third test, sixty hours after stopping cortisone, the response was normal in both subjects. In Subject 1 eosinophil levels were determined also. The first significant drop in eosinophils following the administration of ACTH was observed on the same day that normal steroid response occurred. From the response in the levels of 17-hydroxycorticosteroids, it is apparent that cortisone therapy had induced profound temporary impairment of the adrenocortical capacity in both patients.

DISCUSSION

From the data presented, it is apparent that the determination of 17-hydroxycorticosteroids in plasma from peripheral blood offers an opportunity to study the effect of continuous administration of ACTH upon the adrenal cortex. Adrenocortical function in various disease states can be evaluated when the response of the plasma steroids to the intravenous infusion of 25 I.U. of ACTH over six hours is compared to that of normal sub-

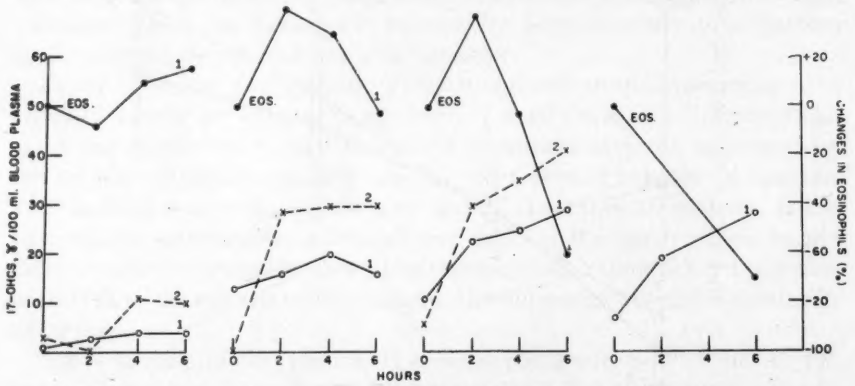

FIG. 4. Plasma 17-hydroxycorticosteroid and circulating eosinophil levels as modified by the infusion of 25 I.U. of ACTH intravenously over six hours on each of four successive days, in patients who had received prolonged oral cortisone therapy. Patient No. 1 had received 200–300 mg. of cortisone acetate daily for twenty-six days. Patient No. 2 had received 150 mg. of cortisone acetate daily for three months.

jects. The clinical conditions in this study were not clearly associated with decreased adrenocortical capacity, except in those instances in which the adrenal cortex was directly involved. Control levels of 17-hydroxycorticosteroids before ACTH administration were not above the upper normal maximum of 22 gamma per 100 ml. of plasma in any of the cases studied, except in the 1 case of Cushing's syndrome. Since blood plasma is obtained from the peripheral circulation, the levels found will reflect both adrenocortical production and corticosteroid metabolism. For the adrenocortical capacity test to be useful in pathologic conditions associated with high levels of plasma 17-hydroxycorticosteroids, it is of paramount importance to establish constancy of the adrenocortical steroid environment beforehand, as in Figure 2. If this environment is fluctuating over the time the test is performed, it is difficult to evaluate the results obtained.

Patients with Addison's disease seem to show a variable picture. As judged by plasma levels of 17-hydroxycorticosteroids, they fall into two groups—cases with measurable levels, and cases with levels not detectable by the method used.

The basis for the symptoms in Addison's disease is lack of adrenocortical hormones. The disease usually develops gradually. Little or no adrenocortical tissue has been found at autopsy in most patients with atrophy, but relatively large remnants have been observed in certain patients with adrenal tuberculosis. If no detectable levels of 17-hydroxycorticosteroids are found in a patient and there is no response upon ACTH administration, a practically complete loss of adrenocortical tissue is indicated. Patients with measurable 17-hydroxycorticosteroid levels, but showing no significant response to the adrenal capacity test, have adrenal remnants which are probably already maximally stimulated by endogenous ACTH. Thus, inadequacy of endogenous ACTH secretion as the cause of the pathologic conditions is ruled out by the test. Patients with normal plasma levels and some response to ACTH, probably have adrenocortical tissue in excess of that which is stimulated maximally by endogenously secreted ACTH. We have observed 2 patients with tuberculous adrenals in whom progression of the severity of the disease was associated with disappearance of circulating 17-hydroxycorticosteroids from the plasma.

In 1 case of Cushing's syndrome a supernormal response of plasma 17-hydroxycorticosteroids to exogenous ACTH was seen, in spite of the high control level. At operation this patient had a large adenoma of the adrenal cortex. As already pointed out, a graded series of subnormal responses is observed in Addison's disease. It would thus appear that the amount of tissue able to produce and secrete adrenocortical hormones is a very important factor in the response to the ACTH infusion test.

Of considerable interest was the apparent rapid return of adrenocortical

capacity in patients who had been receiving prolonged cortisone therapy. Sprague *et al.* (7) found an inactivation of the adrenal cortex in human beings following the administration of cortisone, as judged by the urinary 17-ketosteroid excretion and response of the eosinophils to the administration of ACTH. Normal adrenocortical function, using these criteria as indices, was found from three to twenty days after the cessation of cortisone administration.

In our study, low values for 17-hydroxycorticosteroids were found twelve hours after cessation of cortisone therapy. This could reflect either a functional atrophy of the adrenal cortex following the cortisone administration or an interference with the release or production of pituitary adrenocorticotropic hormone, or both. The rapid return of apparently normal adrenocortical capacity in our 2 patients may indicate that the adrenal depression had not been severe enough to produce serious atrophy. On the other hand, the rapid response perhaps was due to the effects of the previous ACTH tests on the adrenal tissue. Finally, the picture could be explained by a combination of partially restored adrenocortical function and impaired metabolism of corticosteroids induced by the prolonged administration of unphysiologic doses of cortisone. If the ACTH response found within sixty hours after stopping prolonged cortisone therapy reflects normal adrenocortical capacity without alteration of corticosteroid metabolism, it indicates the high flexibility of the gland in adjusting its function to the change in the *internal mileu* and also shows the transient character of the changes induced.

The normal adrenocortical capacity found in pathologic conditions other than adrenal insufficiency and Cushing's syndrome, does not exclude the participation of the adrenal cortex in the development of these diseases. To determine this, it would be necessary to determine adrenocortical capacity throughout the course of the various diseases.

As pointed out in the previous paper (1), this ACTH test can be interpreted critically only when there is a balance between endogenous production and removal, and when the rate of removal can be determined. The latter can be estimated from the rate of disappearance of intravenously injected hydrocortisone (8, 9). The evidence presented indicates that the information thus obtained is more direct, as far as the function of the adrenal cortex in the human is concerned, than that obtained by any method hitherto described.

Because of technical difficulties, the adrenal capacity test probably will not become a routine clinical procedure. Nevertheless, the method has proved of great help in the elucidation of different aspects of adrenocortical function in man. The ACTH adrenal capacity test and the estimation of the rate of removal of intravenous 17-hydroxycorticosterone (8, 9) enable the investigator to estimate adrenal steroid production.

SUMMARY

The response of the plasma 17-hydroxycorticosteroids to a standard ACTH test in various clinical conditions is described. Only in those conditions in which the adrenal cortex was directly affected was a significant change in adrenocortical capacity found. Limitations and uses of the test are discussed.

Acknowledgments

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THE CONCENTRATION OF RELAXIN IN THE BLOOD SERUM AND OTHER TISSUES OF WOMEN DURING PREGNANCY*

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INTRODUCTION

INVESTIGATIONS on the physiology of relaxin would indicate that it is a hormone of pregnancy (1, 2). Additional evidence for this concept may be obtained also from studies on the presence of this hormone in the blood and other tissues during gestation. The concentration of relaxin in the blood of the rabbit (3) and the guinea pig (4) and in the ovary of the pregnant sow increases progressively during pregnancy until a plateau is reached, and then drops precipitously at parturition. In the human being, however, relaxin has been detected in the first part of pregnancy but not in the last trimester (5, 6). Pommerenke (6) reported a drop in the level of serum relaxin of women in the fifth month of pregnancy and a total absence (at 5–6 ml. of serum) by the eighth month.

The present investigation was undertaken to determine quantitatively the blood serum levels of relaxin in women throughout pregnancy and at parturition. In addition, several pregnancies complicated by pre-eclampsia and diabetes were studied.

MATERIAL AND METHODS

Approximately 40 ml. of blood was obtained from each patient, allowed to clot and placed in the refrigerator. After centrifugation the serum was extracted for relaxin, according to the method of Albert and Money (7). The final alcohol-precipitate was dissolved in distilled water and lyophilized for storage. Assays for relaxin were performed on castrated, female guinea pigs primed with estradiol (8). No fewer than 10 guinea pigs were used for each determination and several determinations were carried out on every sample, depending on the response and the amount of serum available. Since there is still no accepted standard for relaxin activity, the sensitivity and reproducibility of the procedure was checked with a standard preparation of commercial relaxin (Releasin).[1]

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[1] Releasin was supplied through the courtesy of Dr. R. L. Kroc, Warner-Chilcott Laboratories.

Several tissues of the reproductive tract were studied for relaxin content. These were ground up in an electric meat grinder or a Waring Blendor, extracted once with 4 volumes and once with 1 volume of 5 per cent sodium chloride and then dialyzed against an equal volume of 95 per cent ethanol. The extract inside the membrane was centrifuged and the supernatant fluid precipitated with 4 to 5 volumes of acetone. The wet precipitate was collected by centrifugation, redissolved in distilled water and lyophilized. An extract of amniotic fluid was similarly prepared by dialysis of 1,250 Gm. of fluid against an equal volume of cold 94 per cent ethanol and subsequent precipitation with acetate.

RESULTS

Relaxin was not detected in the blood of men or of nonpregnant women (Table 1). A minimum of 0.2 G.P.U. (guinea pig unit) per ml. of serum was found at the earliest stage of pregnancy tested, *i.e.*, 7 to 10 weeks. The concentration increased as pregnancy progressed, until it reached a maximal plateau of approximately 2 G.U.P. per ml. during the last few weeks of gestation (Fig. 1). The level fell precipitously at delivery and only about 10 per cent was present twelve hours post partum. No relaxin was detected in blood drawn twenty-four hours after delivery.

TABLE 1. RELAXIN LEVELS IN THE BLOOD SERUM OF MEN AND OF PREGNANT AND POSTPARTUM WOMEN

No. of	Description of patient	Average G.P.U. of relaxin per ml. of serum
2	7–10 weeks of pregnancy	0.2
4	11–18 weeks of pregnancy	0.5
2	20–24 weeks of pregnancy	0.0
5	26–30 weeks of pregnancy	1.2
4	31–33 weeks of pregnancy	1.4
4	35–37 weeks of pregnancy	1.1
2	38 weeks of pregnancy	1.8
2	40–42 weeks of pregnancy	2.0
2	at delivery	1.0
1	12 hours post partum	0.2
1	24 hours post partum	0.0
3	48 hours post partum	0.0
4	men	0.0
2	diabetes (31–32 weeks pregnant)	1.5
2	pre-eclampsia	0.0

The blood samples from 2 pre-eclamptic women were studied in duplicate. In both instances the serum contained less than 0.2 G.P.U. per ml. Since 4 ml. was the minimal amount tested in each guinea pig, this is essentially a zero value. The first patient was in the thirtieth week of pregnancy and aborted a 2-pound baby two days after the sample was obtained. The other

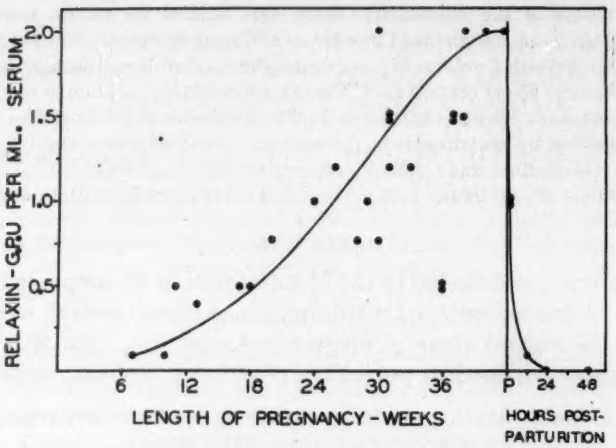

FIG. 1. Concentration of relaxin in the blood serum of normal pregnant women. Note the progressive increase throughout pregnancy and the precipitous drop at parturition.

patient was in the thirty-six week of pregnancy when the blood was examined for relaxin and delivered a normal child four weeks later.

The concentration of relaxin in the blood of the diabetic women tested was 1.5 G.P.U. per ml. at 31 and 32 weeks of pregnancy. These values are essentially normal even though the placentas were greatly enlarged (Fig. 1).

The relaxin content of the placental extracts showed a great deal of variation. Values ranged from less than 1 G.P.U. per gram to 4 G.P.U. per gram-equivalent fresh weight of placenta. Placenta No. 89 was obtained by hysterectomy at the tenth week of pregnancy and showed no activity at the 1 gram-equivalent level. However, in the case of any placental extract, the contribution of the trapped blood in the large sinuses is difficult to evaluate. Amniotic fluid and the uterine myometrium gave negative responses for relaxin (Table 2).

DISCUSSION

Within the limitations of the present bioassay procedure and the limited number of cases, it is clear that relaxin is present in human blood during pregnancy. The rise in titer as pregnancy progresses is similar to that found in the blood of the rabbit (3), the ovary of the sow (8) and to a certain extent the blood of the guinea pig (4). Thus, the general pattern is the same in all species studied so far. Although these data disagree in some respects with those reported by Pommerenke (5) and Abramson, Hurwitt and

TABLE 2. CONCENTRATION OF RELAXIN IN THE PLACENTA AND AMNIOTIC FLUID

Patient	Tissue	Relaxin, G.P.U./Gm.*
HRI	placenta	4
S.C.	placenta	4
HPLI	placenta	neg. at 0.8 Gm.
HPL-2	placenta	0.5
HPL-3	placenta	neg. at 2.2 Gm.
88b	placenta	0.5
89-3	placenta	neg. at 1 Gm.
89-2	amniotic fluid	neg. at 50 ml.

* Refers to the fresh weight.

Lesnick (6), those investigators did not attempt to determine more than the presence or absence of relaxin during the various stages of pregnancy.

The initial work of Hisaw (9) indicated that relaxin was a hormone of pregnancy and most of the functions since attributed to relaxin are related to pregnancy and parturition. Thus, the water-soluble extract of the ovary has been implicated in the development of the mammary gland (10, 11), uterine contractions (12, 13), decidual reaction (14), dilation of the uterine cervix (15, 16) and possibly the anemia of pregnancy (17). That there may be a multiplicity of factors in preparations used for these studies is a probability which should be carefully considered (18), especially since preparations of human sera which relax the pubic symphysis of the guinea pig were not effective in the mouse (19).

The maximum concentration of relaxin in the blood of the pregnant woman, *i.e.*, 2 G.P.U. per ml., is the of same order of magnitude as seen in the pregnant sow (8). This is higher than the levels found in the blood of the guinea pig (4) but is significantly lower than the concentration of 10 G.P.U. per ml. present in the rabbit (3).

It is difficult to state at present the source of relaxin in the woman. The concentration in the placentas varied from zero to 4 G.P.U. per gram-equivalent; this is certainly not comparable to the concentration found in the rabbit, in which the level varied from 250–350 G.P.U. per gram equivalent of fresh maternal placenta (20).

It is of interest to speculate on the therapeutic possibility for this hormone. Numerous unpublished reports have demonstrated that it is ineffective in arthritis. Recent studies have extended this observation and have also indicated that the hormone does not induce any metabolic changes (21). However, in these experiments the physiologic conditioning

with estrogen, found to be necessary for most of the effects of relaxin, was not attempted.

SUMMARY AND CONCLUSIONS

Relaxin appears in the blood of women during pregnancy and increases from a level of 0.2 G.P.U. per ml. of serum at 7 to 10 weeks of pregnancy to a maximum concentration of 2 G.P.U. per ml. at 38 to 42 weeks of pregnancy. The hormone disappears from the blood within twenty-four hours post partum. Seven placentas were analyzed for relaxin. Three contained no relaxin, and the other 5 showed values of 0.5 to 4 G.P.U. per gram equivalent of fresh tissue. A decrease in the concentration of relaxin in the serum was evident in 2 cases of pre-eclampsia.

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EFFECT OF GLUCAGON ON PERIPHERAL UTILIZATION OF GLUCOSE IN MAN*

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GLUCAGON (HGF) is known to produce hyperglycemia by stimulating hepatic glycogenolysis (1, 2, 3). However, the influence of this substance on utilization of glucose by peripheral cells has not been clearly defined (4, 5). De Duve (4) has referred to glucagon as a "powerful insulin antagonist," and has stated that in this regard glucagon is at least as effective as epinephrine. Pincus and Rutman (5) have demonstrated that glucagon can prevent the fall in blood glucose concentration following administration of small doses of insulin. Yet, in order for this protective effect to occur, the animal studied must have been well fed, and the glucagon must be given by repeated injection or continuous infusion. Evidence from other sources (6, 7) also indicates that the hyperglycemic effect of infused glucagon is evanescent and that any effect glucagon might have on peripheral glucose utilization necessarily must be transient in nature as well.

To permit more direct determination of the peripheral action of glucagon with respect to glucose utilization, studies have been performed on normal human subjects in whom peripheral glucose uptake during and after glucagon-induced hyperglycemia was compared with peripheral uptakes during and after similar hyperglycemias produced (a) by intravenous infusion of glucose, and (b) by ingestion of glucose following epinephrine injection.

PROCEDURE

Ten adults without known metabolic disorders served as subjects. All subjects were studied while they were at rest and in the postabsorptive state. For three days prior to each experiment, each subject had been on a high-carbohydrate preparatory diet as recommended by Conn (8). Venous blood samples were obtained without stasis from an antecubital vein, and almost simultaneously, capillary (arterial (9, 10)) blood was collected by direct pipetting from a finger tip of the same extremity after cutaneous puncture. The method of Somogyi (11, 12) as modified by Nelson (13) was used to measure glucose in whole blood. All determinations were performed in duplicate on 0.2-ml. samples.

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The glucagon[1] used in these studies was a highly purified preparation isolated by the method of Staub, Sinn and Behrens (14).

Three types of experiments were performed: (a) Glucagon in saline was administered intravenously by constant (Bowman pump) infusion to all 10 subjects, usually over a 30-minute period. (b) On another occasion, 6 of the subjects were given a constant infusion of glucose in physiologic sodium chloride over a comparable period of time, to simulate the glucagon-induced hyperglycemia. (c) The remaining 4 subjects and 3 of the group who participated in experiment *b* were given a subcutaneous injection of 0.3 mg. of epinephrine U.S.P., and ten minutes later ingested a solution containing 30 Gm. of glucose.

In a separate experiment, 1 subject (A.B.) was given 0.25 mg. of epinephrine intramuscularly, followed ten minutes later by a constant glucagon infusion which lasted approximately sixty minutes.

In every instance, capillary and venous samples were obtained prior to glucagon or glucose administration and at approximately 15-minute intervals thereafter, for two hours.

To permit comparison of the uptakes in the presence of differing capillary glucose levels, relative assimilation indexes (R.A.I.) were calculated for each experiment, according to the formula of Somogyi (15):

$$\text{R.A.I.} = \frac{100 \times \sum(\text{C-V})}{\sum(\text{i})}$$

Where

$\sum$(C-V) = assimilation index = sum of the capillary-venous differences

$\sum$(i) = hyperglycemic sum = sum of the increments in capillary blood glucose over the control level during the corresponding time interval

(Negative increments and their corresponding C-V differences were omitted from the calculations and negative C-V differences associated with positive increments were assigned a value of zero.)

RESULTS

The capillary and venous increments over control values following administration of (a) glucagon, (b) intravenous glucose, and (c) oral glucose plus epinephrine, and the associated capillary-venous (C-V) differences are given in Table 1, along with the R.A.I.'s calculated for each experiment.

For the 6 subjects in whom glucagon and intravenous glucose were compared, the mean R.A.I. associated with glucagon administration was 35.1;

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[1] Glucagon was generously supplied by Dr. F. B. Peck and Dr. W. R. Kirtley of Eli Lilly and Company, Indianapolis, Indiana.

Two independent assays of the insulin content of the glucagon (Lilly #208-158B-34B and #208-158B-214) gave values of 0.05 unit and 0.005 unit of insulin, respectively, per milligram of glucagon. Potency of glucagon was determined by the method of Staub *et al.* (14). In terms of biologic activity, 1 unit was equivalent to approximately 0.15 microgram.

TABLE 1. EFFECT OF GLUCAGON, GLUCOSE AND EPINEPHRINE ON PERIPHERAL ASSIMILATION OF GLUCOSE

Subject	Dose of glucagon (mg.)	Minutes after administration of glucose or glucogon												Hyperglycemic sum	Assimilation index	R.A.I.‡
		15		30		45		60		75		90				
		i*	c-v†	i*	c-v†	i*	c-v†	i*	c-v†	i*	c-v†	i*	c-v†			
Subjects given glucagon intravenously																
A.B.	1.9	51	18	61	28	20§	16	−22	0	−32	−1	− 8	−2	141	62	44.0
A.K.	1.9	25	7	48	17	47	12	37§	20	6	16	−22	5	163	72	44.1
W.G.	1.8	33	12	68	14	52§	8	16	12	−25	0	−16	2	169	46	27.2
H.B.	0.63	38	2	47	6	64	12	79	21	60	20	8	7	296	68	23.0
H.M.	0.38	4	6	15	5	17	6	13	4	2	2	− 3	3	51	23	45.1
D.K.	1.9	30	13	62	35	49	26	30	24	− 9	17	−37	9	171	98	57.3
A.J.	1.7	29	1	64	6	66	21	23	9	− 8	5	−16	2	182	37	20.3
R.B.	1.9	52	2	81	4	93	10	105	18	60	3	25	1	416	38	9.2
J.W.	1.9	44	4	66	9	77	13	78	15	67	8	33	0	365	49	13.4
D.S.	1.7	17	2	33	8	32	15	− 4	4	−15	0	− 5	0	82	25	30.5
										Mean and standard deviation					*31.4± 15.7*	
Subjects given 25 Gm. glucose intravenously																
A.B.	—	45	7	63	17	30	7	8	5	5	−1	7	0	146	36	24.6
H.M.	—	62	28	27	1	5	0	− 6	−1	− 2	1	—	—	94	29	30.9
D.K.	—	19	0	37	17	14	12	− 2	8	− 7	1	− 7	−2	70	29	41.4
A.J.	—	54	5	82	15	43	0	22	9	− 3	0	− 9	2	201	29	14.4
J.W.	—	48	8	66	9	69	10	43	4	23	8	5	1	254	40	15.8
D.S.	—	54	6	74	18	43	10	25	4	7	5	− 1	1	203	43	21.2
										Mean and standard deviation					*24.7± 10.1*	
Subjects given 50 Gm. glucose orally ten minutes after subcutaneous injection of 0.3 mg. epinephrine																
A.B.	—	50	11	61	1	64	4	52	1	37	3	22	6	286	26	9.1
A.K.	—	46	−7	80	−5	112	5	111	3	88	6	51	8	488	22	4.5
W.G.	—	32	3	70	7	94	14	97	8	81	5	53	5	427	42	9.8
H.B.	—	61	7	89	12	68	6	39	3	13	−1	− 7	2	270	28	10.4
R.B.	—	48	0	89	−4	88	−1	81	1	65	−6	55	0	426	1	0.2
J.W.	—	48	0	74	−3	84	−4	77	0	73	0	53	0	389	0	0.0
D.S.	—	12	0	39	1	62	0	66	2	69	4	57	4	305	11	3.6
										Mean and standard deviation					*5.4± 4.4*	

* Capillary glucose increments over control value, in mg. per 100 cc.
† Capillary-venous difference associated with capillary increment, in mg. per 100 cc. (corrected for time).
‡ Relative assimilation index.
§ Falling glucose values during constant glucagon infusion.

in the same subjects the mean R.A.I. associated with intravenous administration of glucose was 24.7; the mean difference was 10.4 The standard deviation of the 6 differences was 7.89 and the standard error of the mean difference was consequently 3.22, giving a t value of 3.23 with 5 degrees of freedom. Reference to the table of t (16) indicates that the probability of exceeding the mean difference by chance alone is between 1 and 5 per cent. The difference thus is of borderline statistical significance.

For the 7 subjects in whom glucagon and oral glucose plus epinephrine were compared, the mean R.A.I. associated with glucagon administration was 27.3; the R.A.I. associated with ingestion of glucose following epinephrine injection was 5.4. The mean difference was 21.9. The standard devi-

ation of the 7 differences was 11.90 and the standard error of the mean difference was consequently 4.49, resulting in a t value of 4.90 with 6 degrees of freedom. Here, reference to the table of t shows that the probability of exceeding the mean difference by chance alone is less than 1 per cent. Hence the difference is statistically significant.

Three individuals (D.S., A.D. and J.W.) served as subjects in all three experiments. The hyperglycemic responses to glucagon, intravenous glucose, and epinephrine followed by oral glucose, all in the same individual (D.S), are shown for purposes of comparison in Figure 1. It is readily apparent that, despite the higher capillary glucose levels achieved during glucose infusion (Fig. 1B), the C-V differences are similar in magnitude to

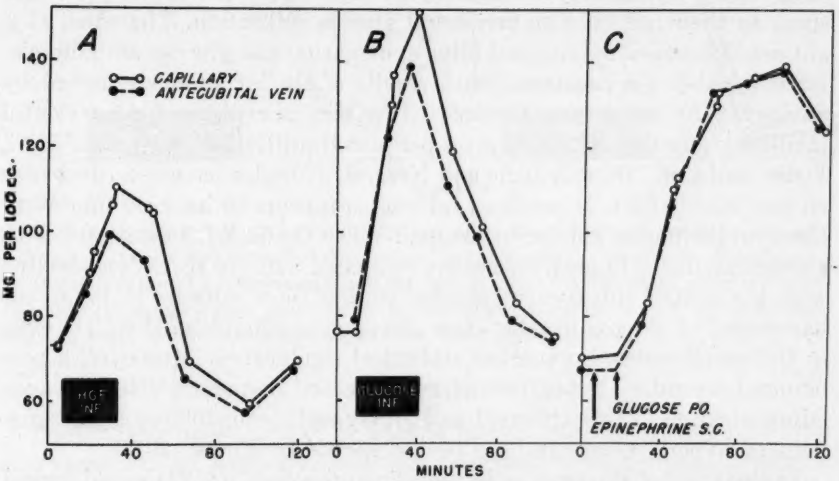

FIG. 1. (Subject D.S.) **A.** Glucagon-induced hyperglycemia with appreciable capillary-venous (C-V) differences maintained on the falling limb of the curve (1.75 mg. of glucagon in 160 cc. of saline given by constant infusion over a 25-minute period). **B.** Hyperglycemia and C-V pattern following constant infusion of 25 Gm. of glucose in physiologic salt solution. **C.** Negligible C-V differences and sustained hyperglycemia following ingestion of 30 Gm. of glucose preceded by subcutaneous injection of 0.3 mg. of epinephrine.

those observed during the glucagon-induced hyperglycemia (Fig. 1A). In the epinephrine-glucose experiment (Fig. 1C), C-V differences are negligible even though an appreciable hyperglycemia occurs.

In 2 subjects (A.B. and A.K.), glucagon administration was prolonged so that the duration of constant glucagon infusion totalled approximately sixty minutes. In these individuals (and in Subject W.G.), both capillary and venous glucose levels actually started to fall and appreciable C-V

differences were maintained while glucagon administration was still in progress. Examples of such a falling curve during glucagon infusion, followed by a secondary hypoglycemia, are shown in Figure 2 (A and B). To the same subject as in Figure 2A, epinephrine was administered intramuscularly prior to constant glucagon infusion. The resulting C-V pattern is shown in Figure 2B; it may be seen that epinephrine effectively diminished C-V differences and accentuated the glucagon-induced hyperglycemia.

DISCUSSION

Comparison of the data (Table 1; Figs. 1 and 2) for the three types of experiment readily discloses the striking difference between the two "hyperglycemic glycogenolytic" agents, glucagon and epinephrine, with respect to their influence on peripheral glucose utilization. The small C-V glucose differences maintained after epinephrine and glucose are administered together are consistent with results of similar studies reported by Somogyi (15), which were interpreted by him as evidence for a powerful inhibitory effect of epinephrine on peripheral utilization of glucose.[2] Cori, Fisher and Cori (18) and Ingle and Nezamis (19) also have described such an inhibitory effect. In contrast, glucagon appears to have no inhibitory effect on peripheral glucose utilization. When the R.A.I.'s associated with glucagon-induced hyperglycemia are compared with the R.A.I.'s associated with the control intravenous glucose studies there appears to be an enhancement of glucose uptake when glucagon is administered which, even in this small series, approaches statistical significance. Moreover, a pronounced secondary hypoglycemia was observed frequently after glucagon administration (Table 1; Figs. 1 and 2). Hypoglycemia following glucagon-induced hyperglycemia also has been observed by Elrick (20).

Designation of glucagon as an "insulin antagonist" (4, 21) would appear to be misleading in view of the fact that no antagonism can be demonstrated between insulin and glucagon action in the periphery. The findings obtained in this series of experiments support the view already expressed by several authors (5, 22, 23) that glucagon, far from being an antagonist to insulin, probably acts synergistically with insulin by making hepatic glycogen stores available for insulin action in the periphery.

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[2] Soskin, Priest and Schutz (17) have questioned the value of arteriovenous differences as indexes of epinephrine influence on peripheral glucose utilization, unless such changes are corrected for changes in blood flow. Somogyi (15), on the other hand, has stated that subcutaneous doses of epinephrine of the order of 0.2–0.3 mg. have insignificant effects on peripheral blood flow in human subjects and cannot by a hemodynamic effect account for the observed changes.

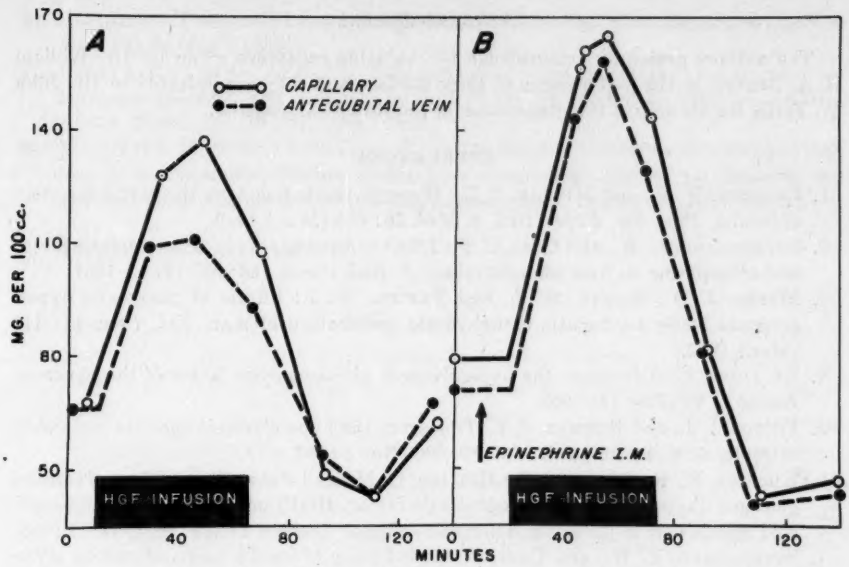

FIG. 2. (Subject A.B.) **A.** Falling C-V curve during prolonged constant glucagon infusion (2.0 mg. of glucagon in saline given over a 55-minute period). **B.** Accentuation of glucagon-induced hyperglycemia and diminution of C-V differences during prolonged constant glucagon infusion preceded by intramuscular injection of 0.25 mg. of epinephrine (1.75 mg. of glucagon in saline given over a 53-minute period).

SUMMARY

Peripheral glucose uptake in normal human subjects during and after glucagon-induced hyperglycemia was compared with peripheral uptakes during and after similar hyperglycemias produced (a) by intravenous infusion of glucose, and (b) by ingestion of glucose following subcutaneous injection of epinephrine. Peripheral glucose capillary-venous differences were determined serially throughout each experiment and served as indexes of glucose uptake by peripheral cells. Comparison of C-V differences following glucagon administration with those following infusion of glucose showed that peripheral utilization of glucose was not inhibited by glucagon. If anything, utilization of glucose may have been enhanced during glucagon-induced hyperglycemia. In contrast, epinephrine injection was followed by apparent inhibition of peripheral glucose uptake.

Since glucagon does not inhibit peripheral utilization of glucose, it is suggested that description of glucagon as an "insulin antagonist" is misleading.

Acknowledgment

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DETERMINATION OF PREGNANEDIOL IN STORED URINE SPECIMENS*

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SEVERAL methods for the determination of pregnanediol in urine have been devised and worked out in detail (1–5). The present communication is the result of the experiences gained with a large number of urine samples, which by force of circumstances had to be stored for long periods of time before they could be analyzed. The known methods of pregnanediol estimation were investigated with regard to their applicability to stored urines, and modifications of these methods were developed which are suitable for the quantitative determination of pregnanediol in urine samples stored for periods up to two years.

METHODS

The urine samples were stored at room temperature under a layer of *n*-butanol. Samples were analyzed by the methods of Venning (1) and Sommerville *et al.* (4) when fresh, and checked after intervals ranging from two to twelve months. The methods of Astwood and Jones (2) and of Talbot (3) were applied also to the stored urines.

The Astwood-Jones method: It was found that some of the pregnanediol was destroyed by this treatment; therefore the procedure was standardized, the loss under the standard conditions determined, and these conditions adhered to strictly when applying this method to an unknown. Occasionally some of the pregnanediol was occluded in the residues from the sodium hydroxide treatment, and in order to avoid losses the toluene suspension was worked up as described subsequently (note IX).

The determination of pregnanediol in stored urine specimens: The method described has been devised with two special conditions in view: 1) The urine samples may be stored before analysis for periods of two years at least, and 2) Estrogens may be determined in the same specimen which is used for the pregnanediol assay. The latter condition requires more drastic acid hydrolysis than would be needed for the determination of pregnanediol only. Pregnanediol is not stable in boiling aqueous acid alone, but the destruction due to the more vigorous treatment becomes negligible if the urine sample is covered with one fifth of its volume of toluene. Of the added pregnanediol, 99 per cent is recovered under the more vigorous conditions of hydrolysis. The method of pregnanediol assay is sketched in the flow sheet and details are given in the following notes.

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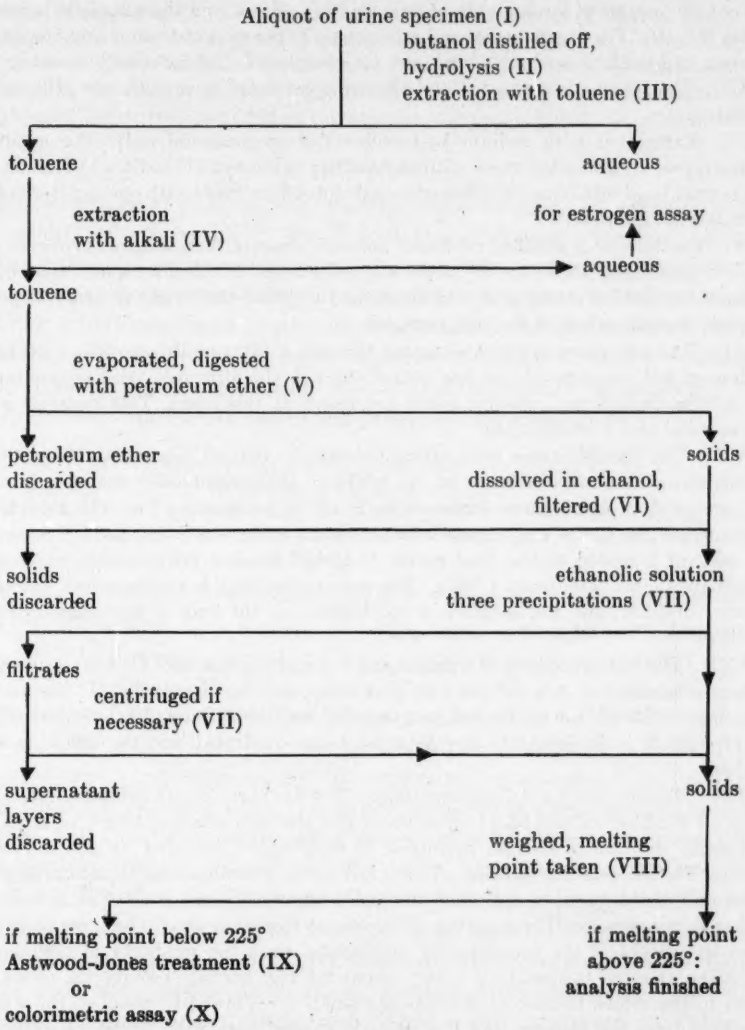
FLOW SHEET



(I). On prolonged storage many urine specimens form a solid deposit, and at the same time the conjugate of pregnanediol is split. The free pregnanediol may be trapped in this deposit if an insufficient quantity of butanol remains with the urine specimen. This may occur when the bottle used to store the specimen is not closed tightly. Therefore, when taking aliquots of urine specimens the sediments should not be neglected if the butanol layer is very small or absent. The butanol must be distilled off before the start of the analysis, since it would interfere with the subsequent separation of the steroids.

(II). Hydrolysis: For pregnanediol only—20 per cent by volume of toluene and 10 per cent by volume of strong hydrochloric acid are added, and the sample is boiled for twenty minutes. For pregnanediol and estrogens—20 per cent of toluene and 15 per cent of strong hydrochloric acid are added, and the sample is boiled for ninety minutes.

(III). The toluene is separated and the urine extracted once with one fifth volume of toluene.

(IV). Extraction with sodium hydroxide: For pregnanediol only—the combined toluene layers are extracted twice with one quarter volume of 1N sodium hydroxide. For pregnanediol and estrogens—the toluene is extracted four times with one quarter volume of 1N sodium hydroxide.

(V). The toluene is distilled off under reduced pressure. The residue is covered with 30 ml. of petroleum ether (b.p. 30° to 60° C.) and allowed to stand for a minimum of two hours. If the residue is oily it should be allowed to stand over night in order to permit complete crystallization of the pregnanediol.

(VI). The petroleum ether is decanted through a filter and discarded. Two 10-ml. portions of hot absolute ethanol are poured through the filter into the flask containing the residue. Sometimes insoluble solids are found at this stage. This material is not pregnanediol and it is discarded.

(VII). The precipitations from ethanol should be carried out exactly according to instructions of Sommerville *et al.* (4). In addition, the pregnanediol resulting from the first precipitation is dried and washed with 10 ml. of petroleum ether. The total loss of pregnanediol due to the treatments with petroleum ether was found to be 1.8 mg. and this amount is added to the final result. If turbid filtrates are encountered they are centrifuged for ten minutes at 1,500 g.[1] The supernatant liquids are discarded, disregarding any turbidity, and the sediment is combined with the bulk of the pregnanediol on the filter.

(VIII). The last precipitate is weighed and if it melts above 225° C. it is considered to be pure pregnanediol. A *pale* brown or pink color may be disregarded. If the material melts below 225° C. the content of pregnanediol may be estimated colorimetrically, or the precipitate is subjected to the Astwood-Jones treatment and the resulting solids weighed.

(IX). Modified Astwood-Jones treatment: The final precipitate obtained (see VII) is taken off the filter in two 10-ml. portions of hot absolute ethanol; 10 ml. of 2 per cent methanolic solution of sodium hydroxide is then added, followed by 100 ml. of dry toluene. The alcohols are distilled off on a hot plate, the sodium hydroxide precipitates at the end of this process, and from this point on, the toluene is allowed to boil for a further thirty minutes. The quantity of toluene at this stage should be more than 2,000 times the weight of the pregnanediol precipitate, since the solubility of pregnanediol in toluene at room temperature is only about 0.5 mg. per ml. Twenty ml. of water is added to the cooled toluene suspension, the solids are allowed to dissolve, the water is separated from the toluene, and the latter is washed once with 20 ml. of water. The toluene is removed under reduced pressure, and the residue taken up in ethanol and precipitated once with water according to the method of Sommerville *et al.* (4). When pure pregnanediol was subjected to this procedure, the recoveries ranged from 89 to 94 per cent; accordingly a correction of 8 per cent is applied when using this method.

(X). The colorimetric determination of pregnanediol: The precipitate obtained as described in (VII) is taken up in ethanol as in (IX); the solution is boiled for two minutes

[1] Gravitational force prevailing at earth's surface.

with a known weight of standardized charcoal and assayed colorimetrically by the method of Talbot *et al.* (3). Very pale solutions require a quantity of charcoal about equal to the weight of the solids present; more strongly colored solutions may need four to five times as much charcoal. The sample of charcoal in use should be standardized by determining the amount of pure pregnanediol adsorbed under the usual conditions and a corresponding correction added to the amount of pregnanediol found colorimetrically.

RESULTS

Determination of sodium pregnanediol glucuronidate: In agreement with the results of others (6) it was found that Venning's method (1) gives higher values for pregnanediol than the methods involving hydrolysis. The rate of spontaneous hydrolysis of sodium pregnanediol glucuronidate (NaPG) which occurs at room temperature in urine covered with butanol is extremely variable; some results are given in Table 1. Urines which have been stored for an appreciable length of time should be analyzed by a method involving hydrolysis of the pregnanediol conjugates.

TABLE 1. PERCENTAGE OF FREE PREGNANEDIOL FOUND IN URINES STORED UNDER BUTANOL

Time stored (months)	2	3	5	11
Free pregnanediol found (%)	3 to 16	14 to 38	12 to 90	47 to 100

Recovery of pregnanediol from its conjugates: The method of Sommerville *et al.* (4) gave fairly pure pregnanediol when applied to fresh urines, and if it was supplemented by the described treatment with petroleum ether (notes V, VI and VII), the final specimens of pregnanediol could be weighed and the colorimetric assay dispensed with. When the same urines were analyzed again several months later, many values obtained were large overestimates, the "pregnanediol" obtained was brown, and its melting point much too low. Treatment of these materials by the method of Astwood and Jones (IX) gave specimens of satisfactory color and melting point, and the values were in agreement quantitatively with those obtained from the urines when fresh. Some results are given in Table 2.

Treatment with petroleum ether: Several contaminants which are known, or are likely to accompany pregnanediol were shown to be removed with petroleum ether. These include cholesterol and pregnan-3-ol-20-one. The loss caused by the treatment with petroleum ether was estimated to be 1.8 mg. in each sample of pregnanediol, if the size of the sample is at least 10 mg. Recoveries of pure pregnanediol added to male urines were at least 98 per cent when allowance for losses was made.

TABLE 2. PREGNANEDIOL CONTENT (MG. PER 24-HOUR SPECIMEN) IN FRESH AND STORED URINES

Sample	A	B	C	D	E	F
Time stored (months)	7	10	16	19	20	24
Initial pregnanediol	29.1	26.2	19.8	24.5	24.2	16.3
Final pregnanediol	31.7	26.6	19.7	22.6	24.8	18.0

Colorimetric and gravimetric estimation of pregnanediol: Several samples of pregnanediol, obtained by the methods described, were weighed and assayed colorimetrically. The purity of the samples as judged by their melting points did not check well with the pregnanediol content as determined by colorimetry, and some of this lack of consistency must be attributed to the colorimetric method. Table 3 shows inconsistencies between samples A, B and C. Probably A is an instance of underestimation by the colorimetric method. At least one of the samples summarized under D showed a gross overestimate. The correction devised by Allen (7) is not applicable to the colorimetric estimation of pregnanediol, the values obtained on using Allen's formula being unreasonably low. The colorimetric method is rendered unreliable by the circumstance that some specimens of pregnanediol contain impurities which develop a color more slowly than pregnanediol does; consequently, the amount of apparent pregnanediol becomes markedly dependent upon the time at which the readings are taken, and it would be exceedingly difficult to apply a correction for this effect. It may be noted in passing that the colorimetric method is not applicable to NaPG, the color obtained being equivalent to only a fraction of the pregnanediol present in the conjugate, even if the sulfuric acid is allowed to act upon the NaPG for twenty-four hours. The crude pregnanediol recovered from stored urines must be treated with charcoal if the colorimetric method is to be used; otherwise, gross overestimates are unavoidable.

TABLE 3. COLORIMETRIC ASSAY OF "PREGNANEDIOL" SAMPLES

Sample	A	B	C	D*
Melting range (°C.)	231–237	225–229	222–230	205–225
Pregnanediol content (%)	88	94	81	84; 92; 168

* These samples had been treated with charcoal, but they were not subjected to the Astwood-Jones purification.

Charcoal treatment: The particular batch of charcoal used in this investigation adsorbed 2.6 per cent of its own weight of pregnanediol under the conditions specified in (X). Therefore, the losses caused by the charcoal treatment are appreciable, especially when 4 to 5 parts of charcoal have to be used for each part of pregnanediol in order to effect adequate decolorization.

Analytical data: The means and standard deviations of the urinary pregnanediol values found in 17 cases of normal pregnancy are given in Table 4. The values are lower than those given by Jones *et al.* (8) and by Michie (9). There are two probable reasons for this: 1) the urine specimens were collected without supervision from patients who were not always very cooperative, and 2) creatinine correction (10) does not allow for losses due to consistently incomplete collections of urine specimens. It is likely also that the treatment with petroleum ether removes chromogenic impurities which were assayed as pregnanediol by previous workers.

TABLE 4. PREGNANEDIOL (MG. PER TWENTY-FOUR HOURS) EXCRETED IN 17 CASES OF NORMAL PREGNANCY

Week of pregnancy	20	22	24	26	28	30	32	34	36	38	40
Mean	13	17	20	23	28	39	36	39	40	37	48
Standard deviation	5	8	8	12	11	13	18	19	14	15	18

DISCUSSION

The results given in Table 4 show the same order of variability as those of other authors, some of whom have commented on the large variations encountered in the 24-hour excretion values for pregnanediol. Much of this variability probably would be eliminated by pooling several "over night" specimens and determining the amount of pregnanediol accompanied by 1 gram of creatinine.

The results obtained show that pregnanediol cannot be determined in stored urine specimens by the original methods of Venning or of Sommerville *et al.* Specimens of crude pregnanediol which had been subjected to digestion with petroleum ether furnished products of a satisfactory degree of purity when they were subjected to the Astwood-Jones purification. The analytical data suggest that weighing of pregnanediol specimens melting above 225°C. is just as reliable as the colorimetric evaluation of these specimens. As an alternative to the Astwood-Jones treatment, sufficient purification for colorimetric assay may frequently be achieved by treat-

ment with adequate amounts of charcoal. Both methods are subject to some degree of uncertainty. Experimental data suggest that the losses involved in the use of these methods are somewhat variable and the corrections can be no more significant than averages obtained from several determinations.

Treatment with petroleum ether: The pregnanediol from pregnancy urine is accompanied frequently by considerable quantities of lipids (11), which invalidate gravimetric determinations; they also interfere with the colorimetric estimations of pregnanediol. These lipids are not eliminated by the conventional methods, but they can be separated from pregnanediol by digestion of the crude material with petroleum ether. If this is carried out at an early stage in the purification, additional practical advantages are obtained. Thus, it permits the use of stopcock lubricant on the separatory funnels; and a drop of butyl phthalate may be added to the original urine specimen in order to prevent foaming when distilling off the butanol which has been used as a preservative. The treatment with petroleum ether facilitates the filtration of the precipitates of crude pregnanediol, and the very turbid filtrates which occur frequently at this stage are eliminated almost entirely.

SUMMARY AND CONCLUSIONS

A modified method is described for the recovery of pregnanediol from human pregnancy urine. This method is applicable to urine specimens which have been stored for periods of two years, using *n*-butanol as a preservative.

The scheme of separation presented provides for the simultaneous determination of pregnanediol and estrogens in a given sample of urine.

Digestion of the crude pregnanediol with petroleum ether is shown to have many advantages in the separation of pregnandiol from urine.

The specimens of pregnanediol may be evaluated either colorimetrically or gravimetrically. Neither method can be relied upon to give good results with all specimens of pregnanediol. A decision regarding the over-all superiority of either method cannot be given at the present time.

Acknowledgment

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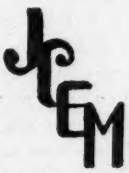

ADULT HYPOTHYROIDISM: REPORT OF AN ADVANCED CASE WITH AUTOPSY STUDY

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THE opportunity to study the morphologic changes of an advanced case of hypothyroidism is seldom obtained. According to Duncan (1) adequate descriptions concerning the pathology of untreated myxedema are to be found only in the older literature. During recent years the utilization of the serum protein-bound determination, the thyroidal I^{131} uptake, and the thyrotropic hormone (TSH) test (2) have aided greatly in the early diagnosis and treatment of myxedema.

In 1944 Foster and Barr (3) recorded the findings in a long-standing case of myxedema, and gave an excellent discussion with respect to the muscular lesions. The scarcity of autopsy cases of adult hypothyroidism has led to the following presentation:

CASE REPORT

Mrs. A. M., R.P.H. #165953, a 68-year-old white housewife, had been confined to a nursing home for two years prior to admission on November 23, 1953. The history was obtained from her son, whose reliability was questionable. For six weeks the patient's relatives had noted progressive mental deterioration, and increasing pallor of the skin.

Past history: There had been a fracture of the right femoral neck at 60 years of age, but otherwise the record was unremarkable.

Physical examination: Temp. 99° F., pulse 60, respirations 18, and blood pressure 160/100. The patient was an obese, 210-pound, elderly white woman, who was disoriented, mentally sluggish, and unable to talk coherently. The skin was pale, scaly and atrophic. The hair was dry, and there was pronounced thinning over the frontal region, The tongue was red and smooth. There was an apical systolic cardiac murmur, and the heart sounds were distant. The abdomen was protuberant, but there were no palpable masses. Pelvic and rectal examinations gave negative results, except for redness and irritation of the external genitalia. There was moderate pitting edema of the ankles. Neurologic examination was difficult because of the patient's mental disorientation. There was generalized weakness and moderate atrophy of the muscles of the extremities. The deep tendon reflexes were hypoactive.

Laboratory data: The urine contained a trace of albumin. The red blood cell count was 3,900,000 with 12.0 Gm. of hemoglobin per 100 ml. The white blood cell count was 4,900 with a normal differential. Blood chemistry studies showed the following: blood sugar, 101 mg. per 100 ml.; icterus index, 12 units; total protein, 6.0 Gm. (albumin, 3.3 Gm.; globulin, 2.7 Gm.); serum alkaline phosphatase, 6.6 units; urea nitrogen, 37

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mg.; serum bromide, 11 mg.; cholesterol, 511 mg.; and serum protein-bound iodine, 2.75 micrograms per 100 ml. (modification of Barker's method; normal value, 4.0–12.0 micrograms) (4).

In view of the slight anemia, the bone marrow was examined. Smears of the sternal marrow showed a moderate hypoplasia of both the erythrocytic and myelocytic elements, with a slight increase in the fat component. There was no disturbance in maturation, and the myeloid-erythroid ratio was within normal limits (5 to 1).

An initial BMR of −28 per cent was recorded. Roentgenograms of the skull showed nothing abnormal. Films of the abdomen disclosed a slight dilatation of the colon and an old ununited fracture of the right femoral neck.

An electrocardiogram showed an inversion of T waves in leads V_3 through V_6, which was suggestive of coronary artery disease. The T waves were of very poor amplitude throughout the tracing, and the latter finding was thought to be consistent with myxedema.

Treatment and course: The case was considered to be one of untreated myxedema, complicated by coronary insufficiency and probable myocardial infarction, and treatment was given on that basis. The patient was started on 1 grain of desiccated thyroid daily, and the dose was gradually increased to 2 grains, and then 3 grains daily. Slight mental improvement was noted, but she was still lethargic. A BMR obtained on the seventeenth hospital day was −17 per cent. In spite of intensive antibiotic therapy, administered prophylactically, bronchopneumonia developed. She became comatose and died on the forty-third hospital day.

Autopsy

The autopsy was performed three hours after death. The significant gross findings were as follows:

The body was that of an obese, elderly, white woman, 95 Kg. in weight, and 162 cm. in length. The obesity of the face, trunk and extremities was especially prominent. Marked thinning of the hair was noted over the entire frontal region. The skin was dry, scaly, and atrophic. The right leg was externally rotated.

The weight of the heart was 450 grams, and there was hypertrophy of the left ventricular myocardium. There was dilatation of all the cardiac chambers, most marked in the left atrial and ventricular chambers. The anterior descending branch of the left coronary artery showed almost complete obliteration of the lumen by atheromatous and calcific plaques. The other major branches of the coronary arteries were focally stenotic, but were patent. There was a recent myocardial infarct, of about ten days' duration, involving most of the anterior portion of the interventricular septum, and the anterior portion of the left ventricle. A large mural thrombus was present in the apical portion of the left ventricular chamber.

The lungs showed moderate pulmonary edema and focal bronchopneumonia. The sigmoid and rectosigmoid portions of the colon were dilated. The kidneys showed changes consistent with arterial and arteriolar nephrosclerosis. There was chronic passive congestion of the other abdominal viscera. The uterus, tubes and ovaries showed involution and atrophy.

The weight of the brain was 1,150 grams. Examination disclosed generalized cortical atrophy, and arteriosclerosis of the major cerebral arteries.

Endocrine glands. The entire thyroid gland was firm, and densely adherent to the surrounding neck structures. Each lobe was small, measured 2.5×1.5×0.8 cm., and weighed 5 grams. The cut surface of the thyroid was firm, gray-white, retracted, and

showed linear zones of fibrosis. The surface was not nodular, but occasional small foci of calcification were noted.

The adrenals were smaller than usual, and had a combined weight of 8 grams. The dimension of each gland was 4×2×0.4 cm. Section revealed a thin, pale-yellow cortex, which averaged 0.3 to 0.2 cm. in thickness. The medulla was of average thickness.

The pancreas was firm, of average size, and weighed 110 grams. The cut surface was lobular, and a homogeneous gray-yellow.

The pituitary was intact and weighed approximately 0.5 gram. There was no evidence of cystic degeneration. The anterior and posterior lobes appeared to be of average dimensions.

Histologic Examination

Thyroid

Representative sections from both lobes of the thyroid showed almost complete replacement of the acini by dense fibrous connective tissue. This was associated with diffuse infiltrations of lymphocytes and large mononuclear cells. Plasma cells and granulocytes were not present in these infiltrations. The few intact acini were mostly of the microfollicular type, and contained little or no stainable colloid. The lining epithelium of the acini was of low cuboidal type, and definitely atrophic (Fig. 1).

Scattered throughout both lobes were occasional clusters of large irregular cells, which showed no tendency to form acini. These cells had small, oval, vesicular, and usually centrally placed nuclei; the cytoplasm was abundant, eosinophilic, and slightly granular. They bore a marked resemblance to oxyphilic, or so-called Hürthle cells (Fig. 2).

Sections of the thyroid were stained with periodic-acid Schiff stain in order to evaluate more accurately the amount of colloid within the gland. This technic showed rare acini containing small amounts of colloid.

Diagnosis. Marked atrophy and fibrosis of the thyroid gland.

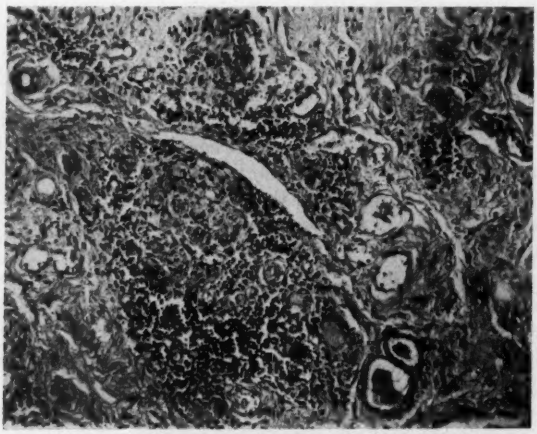

FIG. 1. Thyroid, showing marked fibrosis atrophy and lymphocytic infiltration. Note the clusters of large Hürthle-type cells. ×107, H & E stain.

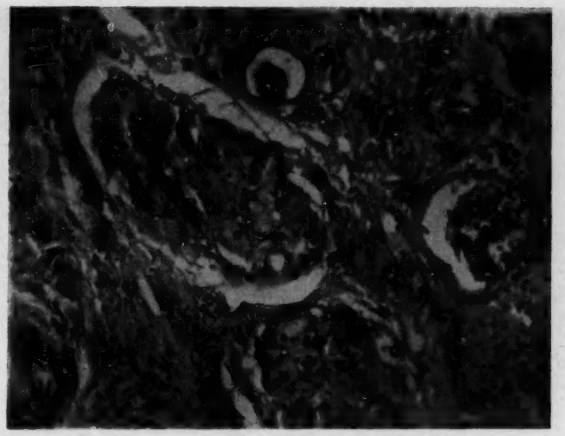

FIG. 2. Details of large oxyphilic Hürthle-type cells present in the thyroid. There is marked atrophy and scant colloid within the few remaining acini. ×267, H & E stain.

Adrenals

The width of the cortex of each adrenal was about one third less than normal. The decrease in number of cells was especially pronounced in the zona fasciculata. Most of the cortical cells showed little or no cytoplasmic vacuolization, indicating lipoid depletion. The medulla of each gland was of average thickness, and the cells were well preserved. Focal infiltrations of lymphocytes were noted throughout the medulla of both adrenals.

Diagnosis. Atrophy and lipoid depletion of adrenal cortex.

Pituitary

Sections of the anterior lobe stained with hematoxylin and eosin showed the following ratio of different cell types: eosinophils 50 per cent, basophils 10 per cent, and chromophobes 40 per cent. The eosinophils were well granulated, but many showed numerous large vacuoles within the cytoplasm. Large, irregular, and bizarre forms of eosinophilic cells were also observed. These cells were much larger than the usual eosinophil, and were characterized by an abundant and granulated, but poorly defined cytoplasm. The nuclei of these cells were small and indistinct (Fig. 3).

Since hematoxylin and eosin staining of the anterior pituitary may give a variable separation of the different cell types, additional sections were prepared with the periodic-acid Schiff stain. The cytoplasm of the large bizarre eosinophils, as well as that of the smaller eosinophilic cells gave a strongly positive reaction with this technic (Fig. 4). The cytoplasmic vacuoles, however, did not give a positive reaction with this stain.

With the routine hematoxylin and eosin stain the basophils showed only small, poorly defined cytoplasmic vacuoles. Rare basophils with nongranulated cytoplasm were noted. The latter cells had a homogeneous, basophilic, and hyaline-like cytoplasm. The chromophobe cells showed no demonstrable changes.

The pars intermedia of the pituitary contained an average number of colloid follicles,

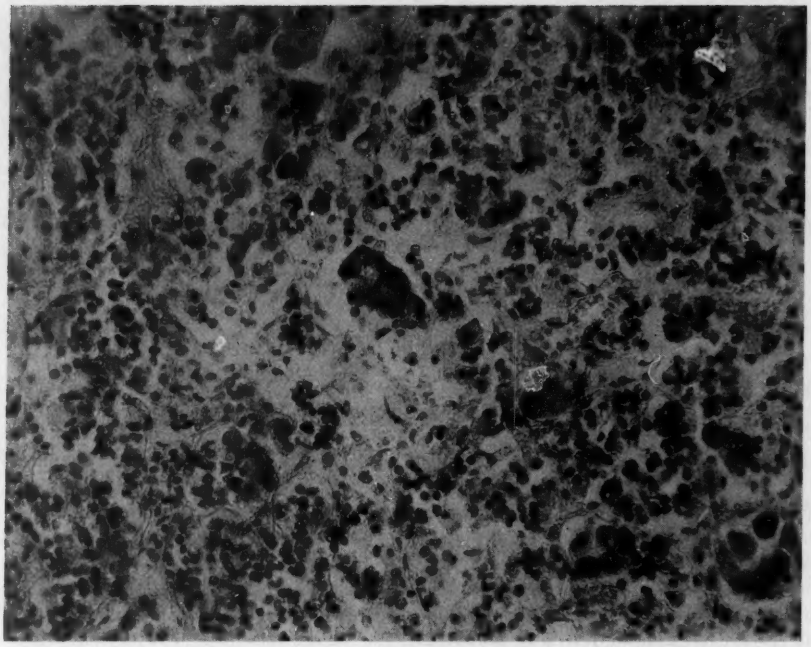

FIG. 3. Anterior lobe of pituitary, showing several large, irregular, and bizarre eosinophilic cells. Smaller, vacuolated eosinophilic cells are also present. ×450, H & E stain.

and the posterior lobe showed no significant deviation from normal. The interstitial stroma throughout the entire pituitary was considered to be within normal limits.

Pancreas

The acinar components of the pancreas were well preserved. There was slight interlobular fibrosis. The islands of Langerhans were normal in number, and did not show degeneration or hyalinization.

Diagnosis. Slight interstitial fibrosis of pancreas.

Heart and aorta

Sections of the interventricular septum and the left ventricular myocardium confirmed the gross impression of a myocardial infarct of about ten days' duration. The smaller coronary arteries showed advanced arteriosclerosis.

Sections of the left ventricle, remote from the infarct, showed peculiar myxoma zones. The individual muscle fibers were fragmented, and vacuolated, and appeared degenerated. Many of the muscle bundles were replaced by a myxomatous and edematous type of connective tissue, which stained basophilic (Fig. 5).

In order to obtain some information with respect to the histochemical nature of the

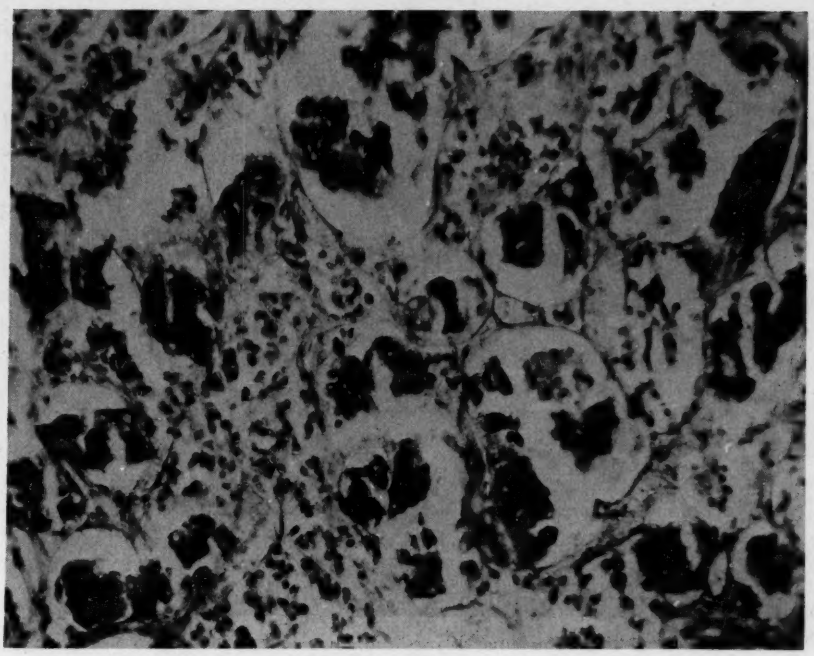

FIG. 4. Anterior lobe of pituitary, showing heavy clumps of PAS-positive material within the cytoplasm of the large irregular cells. Some of the smaller cells are also PAS-positive. ×450, periodic-acid Schiff stain.

peculiar myxomatous zones, sections of the left ventricle were stained with the periodic-acid Schiff stain. The myxomatous zones gave a strongly positive reaction with this technic, indicating the presence of some type of mucopolysaccharide.

Sections of the aorta showed fibrous and hyaline thickening of the intima and subintimal zones. The smooth muscle bundles of the media were fragmented slightly, but did not show the marked degenerative changes observed in the cardiac musculature.

Striated muscle and skin

Sections of the sternocleidomastoid muscle showed fragmentation and vacuolization of the individual fibers. Some of the muscle was replaced by a basophilic edematous material, similar to that found in the left ventricle.

In sections of skin from the lower abdomen and upper thigh there was atrophy of the epidermis, and almost complete lack of hair follicles, sweat and sebaceous glands. The corium was edematous, myxomatous, and basophilic in staining properties. Focal infiltrations composed exclusively of lymphocytes were also present in the corium (Fig. 6).

The myxomatous zones in the striated muscle and in the corium of the skin also gave a strongly positive reaction with the periodic-acid Schiff stain.

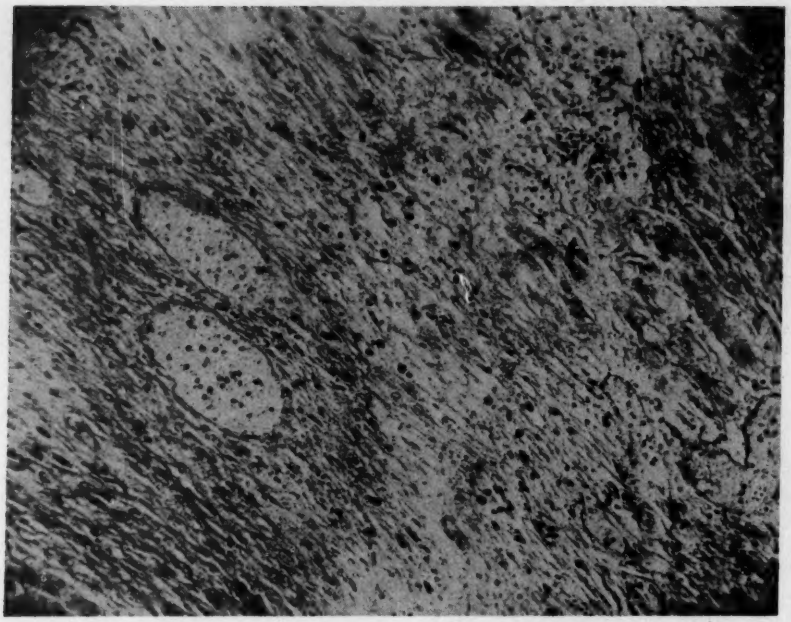

FIG. 5. Muscle of left ventricular myocardium, showing pronounced vacuolization of muscle fibers. Note the edematous and myxomatous change in the interstitial tissue. ×160, H & E stain.

Smooth muscle

No abnormalities were noted within the smooth muscle coats of the gastrointestinal tract, uterus, or urinary bladder. The smaller arteries throughout all the organs showed no detectable changes.

DISCUSSION

According to Shuman (5) hypothyroidism may result from either a primary defect in the thyroid, or from failure of the anterior pituitary to stimulate the usual function of the thyroid. When hypothyroidism is due to failure of the thyroid it is usually called primary, whereas when it results from tumor or destruction of the pituitary it is considered secondary (pituitary myxedema). In the latter type, changes may or may not be demonstrated in the adrenals and other endocrine organs.

It is difficult to estimate the duration of the hypothyroid state in the case presented here, mainly because of the inadequate clinical information. The morphologic findings duplicate, with few exceptions, the changes described by Foster and Barr (3). In their case, the hypothyroidism was thought to have existed for about twelve years.

The morphologic changes in the anterior pituitary in untreated cases of myxedema in adults are not well established, due to the scarcity of such case reports. Anderson (6) states that grossly the pituitary may be either normal, enlarged, or diminished in size. The histologic picture is even more confusing. Proliferation of chromophilic cells, proliferation of chief cells (chromophobes) only, and proliferation of all cell types with chief cells predominating have been described.

Burt (7), by the application of the periodic-acid Schiff (PAS) technic to the anterior pituitary found a significant increase in the sparsely granulated PAS-positive type of cell in various altered physiologic states (*e.g.*, thyroid aplasia, following thyroidectomy, and following propylthiouracil or I^{131} treatment). Laqueur (8), using essentially the same methods, found anterior lobe changes suggesting increased activity of the beta cells in myxedema, and in a small number of individuals with severe lipoid depletion of the adrenal cortex.

Whether the foregoing changes, and the changes described in the present case, are constant remains to be substantiated by further case reports. Apparently the histologic picture of the anterior lobe in untreated myxedema may be quite variable.

Foster and Barr (3) described myxomatous degeneration not only of the heart and striated muscle, but also of the smooth muscle of the esophagus,

FIG. 6. Skin, showing the peculiar myxomatous change throughout the corium. There is a sparse lymphocytic infiltration just beneath the basal layer of the epidermis. ×107, H & E stain.

sigmoid colon, gallbladder, urinary bladder, and uterus. Kountz (9) has emphasized the occurrence of medial degeneration of the aorta and arteries in patients with surgically induced hypothyroidism. Such changes were not present in our case.

Qualitative histochemical studies with the periodic-acid Schiff technic tend to indicate that the peculiar degeneration and myxoma-like edema within the skin, cardiac and striated muscle are related to the presence of a mucopolysaccharide. The elaboration of some type of acid mucopolysaccharide in patients with myxedema has been noted previously by Altshuler and Angevine (10), as well as others.

The bone marrow examination may occasionally prove to be a supplementary aid in the diagnosis of hypothyroidism. Axelrod and Berman (11) found that the functioning marrow is decreased, and usually hypoplastic in hypothyroidism. The myeloid-erythroid ratio is usually normal, but the hypoplasia affects all myeloid systems about equally. The results of the sternal marrow examination in our case agree essentially with those described by the latter authors.

Bruger and Rosenkrantz (12) as well as McGavack and Schwimmer (13) have pointed out the high incidence of cardiac complications in patients with hypothyroidism. The immediate cause of death in the case presented here was most likely myocardial insufficiency secondary to marked coronary arteriosclerosis, and a recent myocardial infarct. It is not unlikely, however, that the myxomatous changes within the myocardium, directly attributed to the hypothyroidism, played some role in the myocardial insufficiency.

SUMMARY

A case of primary hypothyroidism occurring in an adult woman is discussed. Morphologically the condition was characterized by fibrosis and atrophy of the thyroid gland, and by myxomatous changes in cardiac and striated muscle, and the corium of the skin. Associated findings were atrophy and lipoid depletion of the adrenal cortex.

The variable histologic pattern in the anterior pituitary in primary hypothyroidism is discussed briefly.

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THE METABOLISM OF IODINE IN 2 GOITROUS CRETINS COMPARED WITH THAT IN 2 PATIENTS RECEIVING METHIMAZOLE*†

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CONGENITAL hypothyroidism which is commonly seen in the iodine-deficient areas of the world is known as *endemic cretinism*. The disorder, which results from depletion of iodine in the diet of successive generations, may be accompanied by atrophy and degeneration of the thyroid gland, or by hyperplasia, fibrosis, and cyst formation (1). The pathologic end-result evidently depends upon the degree of deprivation of iodine.

The type of cretinism customarily encountered in regions where goiter is not endemic is known as *sporadic cretinism*. Subjects of this disease may harbor a remnant of atrophic thyroid tissue, but often not a vestige of thyroid is detectable. Either the gland is congenitally absent, or the physiologic stimulus from the anterior pituitary fails for reasons which are not known.

Cretins with goiter are rarely encountered in nonendemic regions. Lerman *et al.* (2) in 1946 described 2 goitrous brothers who had juvenile myxedema. The thyroid of one of these had a high uptake of I^{131}-labeled iodine and, according to an analysis of a biopsy specimen, contained a low concentration of iodine. In the other, uptake was normal.

Stanbury and Hedge (3) reported 4 siblings who were pronounced cretins with large goiters, and later added a further case from another family (4). The thyroidal uptake of I^{131}-labeled iodine was rapid and high, but when

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large oral doses of thiocyanate were also given, the labeled iodine retained by the thyroid was almost quantitatively discharged. This observation indicated that the metabolic defect of these huge glands was their inability to convert trapped iodide into an organically bound form. The goiter was apparently the result of a compensatory effort to relieve the deficiency resulting from failure of hormone synthesis.

More recently, Hubble (5) has reported 4 hypothyroid siblings with goiter. In one of these, I^{131}-labeled thyroxine was demonstrated both in the gland and in the serum twenty-four hours after a tracer dose of I^{131}; and in another, an elevated uptake of labeled iodine was observed. McGirr and Hutchison (6) have also described their observations on 12 cases of nonendemic goitrous cretinism. Several of these cretins were siblings or close relatives. All showed a normal or high uptake of labeled iodine and, of 9 tested, all showed protein-bound *labeled* iodine in the peripheral blood at forty-eight hours. One blood sample which was studied by column chromatography contained a butyl-alcohol soluble component which was neither thyroxine nor triiodothyronine. Wilkins (7) also observed goitrous cretins with normal or high uptakes of I^{131}-labeled iodine. The iodine appeared to be firmly bound to thyroid protein, but its chemical nature was not identified.

In an endeavor to elucidate the disorder of iodine metabolism encountered in goitrous cretins, studies of the metabolic fate of iodine in 2 such patients have been conducted, and the results compared with similar observations made upon 1 patient with Graves' disease and 1 with a normal thyroid gland, both of whom were receiving methimazole (1-methyl-2-mercaptoimidazole). The thyroid glands from the 2 cretins were obtained for analysis.

METHODS

Measurements of uptake of I^{131}-labeled iodine were made both by a collimated single-channel scintillation detector placed in front of the gland, and in a four-channel scintillation array (8). The labeled iodine of urine was measured in a well-type scintillation crystal which had a sensitivity of approximately 10^6 counts per minute per microcurie (cpm/μc.) Samples of serum were measured similarly. Chemical assays of iodine in serum, urine and tissue were performed by a modification of the method of Barker (9). The proportions of iodine and of labeled iodine present in the serum in organic combination were determined by measurements of the precipitate and of the supernatant liquid after precipitation with 5 per cent trichloracetic acid. Slices of tissue weighing 0.5 to 1 gram were homogenized in a Potter homogenizer and precipitated with trichloracetic acid. The precipitate and supernatant liquid were assayed for stable and for labeled iodine. Methods for the chromatographic analyses of blood and tissues (10) and for the determination of thyroxine in tissue (11) have been previously described.

EXPERIMENTAL PROCEDURES AND RESULTS

Case 1, R. E., M.G.H. #357136

A 17-year-old male cretin with 3 goitrous and cretinous siblings was Case 3 of a previous report (3). Details of early history were obscure. A diagnosis of cretinism had been made at the end of the first year of life, and desiccated thyroid had been given intermittently thereafter. He had been studied four years previously on the metabolic wards. At that time the protein-bound iodine concentration in the serum was 1.4 micrograms per 100 cc. The excretion of iodine in the urine varied from 86 to 232 micrograms per twenty-four hours. The patient had received no thyroid medication for at least two months before the present admission.

The height was 142 cm. and weight 40.6 Kg. He was able to talk in the simplest terms and to feed himself. He was not incontinent. Results of routine examinations of blood and urine were within normal limits. The concentration of protein-bound iodine in the serum was 0.9 microgram per 100 cc. Temperature, pulse and respirations were normal. The blood pressure was 86/54. The skin was cool, coarse, dry and loose, and had a dusky, yellowish tinge. The nose was broad and flat, and the lips were full and slightly protruding. The tongue was enlarged, and the voice was husky. The thyroid gland was fully five times normal size and was multinodular. The right lobe was larger than the left. A systolic murmur was heard over the gland. The abdomen was protuberant but there was no hernia. The phallus was small; the right testis was incompletely descended, but the left seemed normal. At the completion of the studies to be described, the patient underwent a bilateral thyroidectomy and had an uneventful convalescence. Microscopic examination of the excised gland disclosed intense hyperplasia.

From previous studies of this patient (3) it was surmised that all the labeled iodine present in the gland was in the form of iodide held in preferential concentration by the thyroid cell, but in free and reversible equilibrium with the iodide of the blood. To test further this hypothesis, the patient was given 200 μc. of I^{131} intravenously. Investigations included the *in vivo* disappearance of the labeled iodide from the gland and blood, and the appearance of labeled iodide in the urine. Ten days later, and twenty-four hours after an 800-μc. tracer of I^{131}, the entire thyroid gland was removed and analyzed for the presence of labeled substances as well as for total and organically bound unlabeled iodine.

Retention curve: The results of the dynamic study are shown in Figure 1. Maximal accumulation of labeled iodide was observed almost immediately after injection. The counting rate over the gland extrapolated to zero time corresponded to 68 per cent of the administered dose. The quantity of labeled iodide in the gland declined at a rate estimated at 2.51 per cent per hour ($T\frac{1}{2} = 27.6$ hours)[1] for the first few days of observation, and

[1] The half time, $T\frac{1}{2}$, is related to the rate constant K by the expression:

$$T\tfrac{1}{2} = \frac{0.693}{K}$$

(0.693 is the natural logarithm of 2.)

then appeared to approach an exponential fall at a rate of 1.92 per cent per hour ($T\frac{1}{2} = 35.9$ hours).

Iodide compartment: The labeled iodide of the blood was measured frequently for the first few hours after administration and daily thereafter. After a few hours the disappearance of labeled iodide from the blood followed a simple exponential having a rate constant of 1.92 per cent per

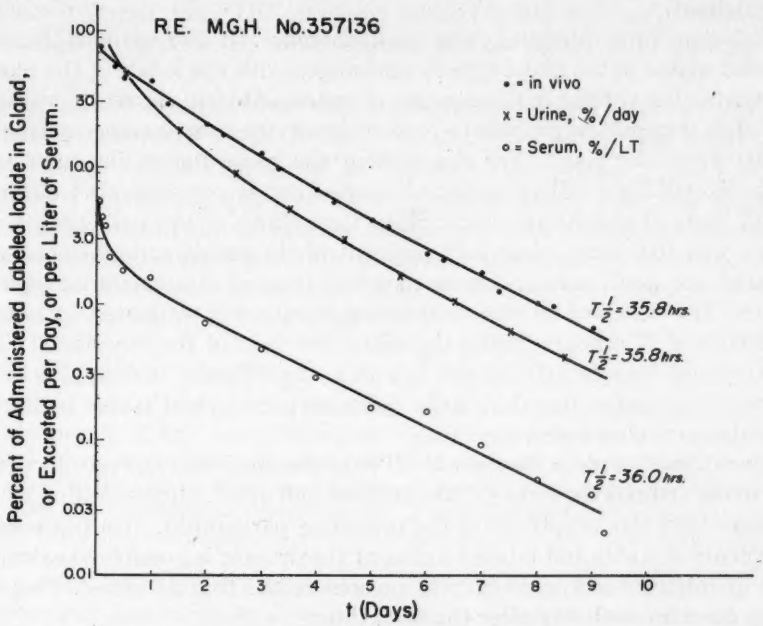

FIG. 1. *Case* 1. The metabolic fate of labeled iodine in a 17-year-old goitrous cretin The tracer dose of I^{131} was given intravenously at zero time. The abscissa is in days. The ordinate is in percentage of dose in gland, urine, and serum.

hour ($T\frac{1}{2} = 1.5$ days). The extrapolated concentration of iodide in the blood at zero time was 2.5 per cent per hour. The volume of distribution was therefore 40.0 liters. Since 68 per cent of the administered dose was present in the gland at zero time, it is evident that the iodide compartment of the thyroid accounted for 27.2 liters, and that the extrathyroidal iodide compartment was 12.8 liters, or 0.31 liter per kilogram of body weight. This compares well with the figures given by Ingbar (12) for normal subjects and patients with a variety of diseases.

Urinary excretion: The urine was collected in half-hourly fractions during the first few hours following administration of the labeled iodide, and

thereafter in daily quantities. The initial excretion rates calculated on the basis of percentage of administered dose of labeled iodide per twenty-four hours were high because of the sweep of labeled iodide through the kidney before equilibration had occurred throughout the iodide compartment, but thereafter the daily excretion of labeled iodide fell along a simple exponential curve closely paralleling those of the blood and urine. The proportion of gland content excreted per day was 5.15 per cent and was constant throughout.

Retention curve predicted from renal clearance: If one assumes that the labeled iodine in the gland rapidly exchanged with the iodide of the blood, then from the volume of distribution of iodide and from the renal clearance of iodide it should be possible to predict the *in vivo* disappearance of labeled iodine from the gland. For this patient the mean iodide clearance rate from six half-hour collection periods carried out at two separate times was 1.315 liters of plasma per hour. Since the volume of the iodide compartment was 40.0 liters, then 3.29 per cent of the iodide compartment was cleared per hour, corresponding to a half-time of disappearance of 21.1 hours. The observed *in vivo* disappearance rate was estimated to have a half-time of 27.6 hours during the initial few days of the experiment. The discrepancy may be attributable in part to the difficulty in obtaining satisfactory urine collections during the clearance periods, and in part to diurnal variations in clearance rates.

Quantity of iodide in the thyroid: If it be assumed that the specific activity in the gland is the same as that in blood and urine (an assumption which follows from the hypothesis of the preceding paragraph), then from measurements of stable and labeled iodine of the urine it is possible to calculate the quantity of iodine which was present in the thyroid gland. This has been done for each day after the first, thus:

$$Qg = \frac{Qg^* E}{E^*} \qquad (I)$$

where Qg^* is the percentage of the administered dose of labeled iodine in the gland, E the 24-hour excretion of iodide and E^* the 24-hour excretion of labeled iodide. The mean value for Qg calculated for ten days was the astonishingly small quantity, 257 micrograms.

The total iodine content of the gland was measured directly by chemical analysis of three fragments of approximately 0.5 gram each, from three separate areas. Each fragment was homogenized and precipitated with trichloracetic acid. The iodine contained in the precipitate and in the supernatant fluid was assayed chemically. Over 99 per cent of the iodine was present in the supernatant layer. The weighted mean content was 3.89 μg. per gram of wet tissue. The excised gland weighed 82.2 grams. The gland

content of iodine therefore was 319 μg., which agrees well with the calculated estimate of iodine content of the whole gland.

The labeled iodine content of three tissue fragments adjacent to those just described was measured. The tissue was homogenized and precipitated with trichloracetic acid. Less than 1 per cent of the labeled iodine was present in the precipitate. The gland content of labeled iodine calculated from the weighted mean content of the three fragments was 32.3 per cent of the administered dose. The measured labeled iodine content of the whole excised gland immediately after surgery, 36.4 per cent, was in close agreement.

Analysis of blood and tissue: Chromatographic analyses were performed on four fragments of tissue. In each case abundant iodide was found, but in no case was there evidence of any iodinated compound. Under similar conditions the normal gland or the hyperplastic gland of Graves' disease contains an abundance of labeled monoiodotyrosine, diiodotyrosine and thyroxine. Chromatographic study of blood drawn twenty-four hours after the labeled iodide was given also failed to disclose the presence of any labeled substance other than iodide.

Case 2, J.V.G., M.G.H. #39256

The observations made on this subject served as a control for those made on the previous patient. J.V.G. was a 36-year-old unemployed male who had entered the hospital several weeks previously for regulation of his poorly controlled diabetes. Two admissions to the hospital seven years previously were for diabetic acidosis. At no time was there any suggestion of thyroid disease. A complicating factor was mental deficiency. Under hospital management it proved possible to control his diabetes without undue difficulty, following which he consented to undergo the observations now to be described. The findings on physical examination at the time of his transfer to the metabolic ward were essentially normal. There was no clinical evidence of either hyperthyroidism or hypothyroidism. The thyroid gland could be palpated but was of normal size, contour and consistency. He required 50 to 60 units of insulin daily. The protein-bound iodine concentration of the serum was 5.1 micrograms per 100 cc. at the beginning of the period of observation, and 4.6 micrograms at the end.

Retention curve: After several days' residence on the metabolic ward, the patient was started on methimazole, 30 mg. every eight hours. On the following day he was given 900 μc. of I^{131} intravenously. Measurements similar to those of the previous patient were made, excepting that the thyroid gland was not removed. Maximal counts were recorded almost at once over the gland (Fig. 2). The subsequent disappearance rate of labeled iodine from the gland was 5.72 per cent per hour ($T\frac{1}{2} = 12.1$ hours). After forty-eight hours, less than 1.5 per cent of the labeled iodine remained in the gland.

Iodide compartment: The labeled iodine content of the blood fell after

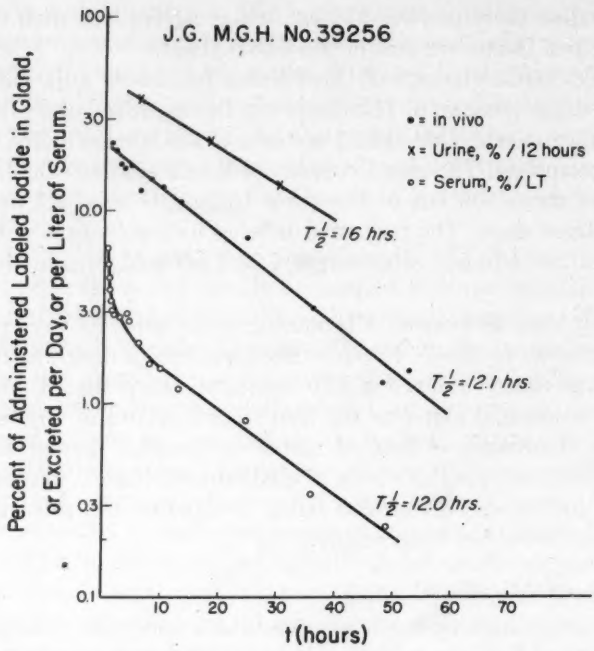

FIG. 2. *Case* 2. The metabolic fate of labeled iodine in a 36-year-old subject with normal thyroid function. Methimazole was given throughout the study, and the tracer dose of I^{131} was given intravenously at zero time. The construction of the chart is similar to that of Figure 1.

an initial period of equilibration at a rate of 5.77 per cent per liter ($T\frac{1}{2}$ = 12.0 days). The extrapolated concentration of labeled iodide was 3.4 per cent per liter, giving a volume of distribution of 29.4 liters. The extrapolated maximal gland content of labeled iodine was 23 per cent of the administered dose. The iodide space of the gland was therefore 6.8 liters, and that of the extrathyroidal compartment, 22.6 liters, or 0.376 liter per kilogram of body weight.

Urinary excretion: Urine collections were incomplete after the second day of collection. The half time of sixteen hours, therefore, is based on only three points. A renal clearance rate of iodide determined during the first few hours of the experiment gave a mean rate of 32.5 cc. per minute for five half-hourly periods. Calculation of the expected *in vivo* decay rate based on renal clearance, size of the iodide compartment, and based on the hypothesis that all the labeled iodine in the gland was in inorganic form, gave a value of 6.63 per cent per hour ($T\frac{1}{2}$ = 10.5 hours). The observed half-time was $T\frac{1}{2}$ = 12.1 hours.

Case 3, McG., M.G.H. #841547

A 49-year-old tax collector was referred to the hospital because of shortness of breath, weight loss, tremulousness, prominence of the eyes, irritability and palpitation. He had been given pills to take but had discontinued their use several months earlier. Physical examination disclosed the typical signs of moderately severe Graves' disease. The skin was warm, moist, and flushed. There was tremor of the extended fingers and a stare. The blood pressure was 134/68 mm. of Hg and the pulse was regular at 100 beats per minute. The thyroid gland was approximately twice the normal size, diffusely enlarged and moderately firm; it moved well with swallowing. The rest of the physical examination showed nothing abnormal. The basal metabolic rate was +47 per cent, and the protein-bound iodine concentration of the serum was 13.6 micrograms per 100 cc.

Retention curve: The patient was started on methimazole, 30 mg. every eight hours, after he had resided on the metabolic ward for several days. One day later he was given 1 millicurie of I^{131} intravenously. Measurements (the same as those performed in Case 2) are shown in Figure 3. *In vivo* uptake rose rapidly to a maximum of 55 per cent in less than two hours, then fell rapidly for two days. Thereafter the decrease was much less rapid and

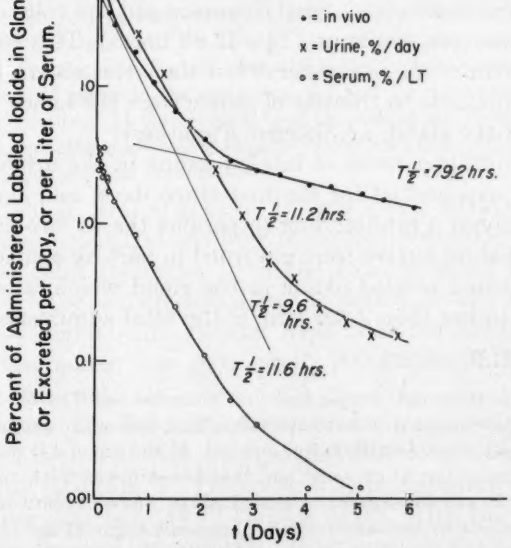
Mc.G. M.G.H. No.841547
• = in vivo
x = Urine, % / day
o = Serum, % / LT
Percent of Administered Labeled Iodide in Gland, or Excreted per Day, or per Liter of Serum.
100
10
1.0
0.1
0.01
$T\frac{1}{2}$ = 79.2 hrs.
$T\frac{1}{2}$ = 11.2 hrs.
$T\frac{1}{2}$ = 9.6 hrs.
$T\frac{1}{2}$ = 11.6 hrs.
0 1 2 3 4 5 6
t (Days)

FIG. 3. *Case* 3. The fate of labeled iodine in a 49-year-old male with Graves' disease. Methimazole was given throughout the study. The I^{131} was given intravenously at zero time. The exponential with a half-time of 9.6 hours was obtained by subtraction of the line drawn through the last seven points of the curve from the earlier points of that curve.

appeared to be approaching an exponential fall with a much slower release rate, having a half-time of 79.2 hours. By subtraction, the first exponential of release was obtained. This had a rate constant of 7.2 per cent per hour ($T\frac{1}{2} = 9.6$ hours). Extrapolation of this component to zero time gave a thyroid content of labeled iodine of 50 per cent for this compartment of the thyroid.

Iodide compartment: After a rapid period of equilibration the labeled iodine of the blood disappeared with an approximate half-time of 11.6 hours for the first two days, but then appeared to be assuming a slower rate of disappearance. Unfortunately, the counts were so low that it was impossible to establish a slope for the second component with any accuracy. The extrapolated concentration of labeled iodide in the blood was 2.5 per cent per liter at zero time. The iodide compartment therefore was 40.0 liters, that of the gland 20.0 liters, and that of the extrathyroidal space 20.0 liters or 0.289 liter per kilogram of body weight.

Urinary excretion: The renal clearance of iodide was measured during the first few hours after administration of labeled iodide. The mean rate for eight half-hourly periods was 35.6 cc. per minute. The *in vivo* curve of disappearance of labeled iodide from the gland attributable to renal excretion alone, and calculated from renal clearance and the volume of distribution, was 5.34 per cent per hour ($T\frac{1}{2} = 12.98$ hours). This corresponds to the first component of the *in vivo* curve but the latter also included a small component attributable to transfer of iodide from the iodide to the iodine compartment of the gland, *i.e.*, hormone synthesis.

The curve of disappearance of labeled iodine in the urine appeared to follow a simple exponential for the first three days, and then to decline much more slowly at a rate tending to parallel the *in vivo* curve and the blood curve. All three curves were governed in part by a small component of organically bound labeled iodine in the gland which, after the fourth day, amounted to less than 2 per cent of the total administered dose.

Case 4, M. D., M.G.H. #237875

This 25-year-old unmarried female had first attended the Thyroid Clinic fourteen years previously. She was said to have started talking before the age of 1 and to have started walking at 2½ years. Dentition was delayed. At the age of 4 it was observed that she was not growing as fast as expected and that her skin was thick and dry. She was sensitive to cold and was constipated. Although there was an improvement when thyroid was given, medication was intermittent. Catamenia began at age 15. Subsequently, menses were present but irregular. At age 17, in spite of a normal metabolic rate on a daily dose of 0.1 Gm. of thyroid, it was observed that her thyroid gland was enlarging. A measurement of her intelligence quotient at age 18 gave a value of 66. Three and one-half years before the present admission the patient was studied for a short time on the metabolic ward. The protein-bound iodine concentration of the serum was 3.5 micrograms per 100 cc. The daily excretion of I^{127} varied from 111 to 190 micrograms per

twenty-four hours. These tests were made at least eight weeks after her last ingestion of desiccated thyroid. Urine specimens collected at home contained similar quantities of I^{127}. The patient continued to feel fairly well with no thyroid medication, and determinations of the basal metabolic rate continued within the normal range. Another determination of serum protein-bound iodine was 5.3 micrograms per 100 cc.

The family history was of particular interest because an older sister suffered from an identical disorder. The sister, now aged 29, was also seen in the Thyroid Clinic fourteen years previously, when the present patient was attending for the first time. The sister's dentition and early development had also been delayed and, in spite of thyroid medication, her thyroid had increased slowly in size. A determination of the intelligence quotient gave a reading of 76. Bone age in Patient M.D., as in her sibling, corresponded with chronologic age. The excretion of I^{127} in the urine was found to be normal, but in both patient and sister an extremely rapid and high uptake of I^{131}-labeled iodine was observed. The protein-bound iodine concentration of the serum of the sister was 6.1 micrograms per 100 cc. Thiocyanate in both sisters failed to discharge any of the retained labeled iodine from the gland. These tests had been performed at least six weeks after the last administration of thyroid. Because her gland was enlarging rapidly in spite of medication with desiccated thyroid, the sister of the present patient underwent an uneventful subtotal thyroidectomy. The pathologic finding was multinodular goiter with extreme hyperplasia. There was no colloid formation within the follicles. She was subsequently maintained on desiccated thyroid.

On physical examination the patient was pleasant but extremely shy. Her arms and legs were disproportionately short. Her height was 148.5 cm. and her weight 54.06 Kg. The skin was cool and dry and the hair was coarse and dry. The thyroid was very large, the right lobe being larger than the left; it was multinodular and moderately firm. Otherwise, the findings on examination were within the limits of normal. The concentration of protein-bound iodine in the serum was 6.2 and 5.4 micrograms per 100 cc. on successive determinations. At the completion of the studies she underwent a total thyroidectomy. Her convalescence and subsequent course were uneventful. Extreme hyperplasia was noted on histologic examination.

The initial 200-μc. tracer dose of I^{131} was given intravenously. Blood, urine, and *in vivo* concentrations were measured frequently during the first few hours and daily thereafter until the twenty-second day. From the eleventh through the seventeenth days the patient received 30 mg. of methimazole every eight hours. Several days after the completion of these observations the patient was given orally 900 μc. of I^{131}. Four days later the gland was removed.

Retention curve: The labeled iodine accumulated very rapidly in the thyroid to a maximum of 99.3 per cent and thereafter slowly disappeared at a diminishing rate (Fig. 4). It appeared to be approaching a rate of release of 2.96 per cent per day ($T\frac{1}{2} = 23.4$ days). With the administration of methimazole, the rate increased to 7.3 per cent per day ($T\frac{1}{2} = 9.5$ days),[2] presumably because of inhibition of re-utilization of labeled iodide. When methimazole was withdrawn, the rate of release became slower.

[2] These slopes were calculated by the method of least squares. The change in slope was significant ($P < 0.05$).

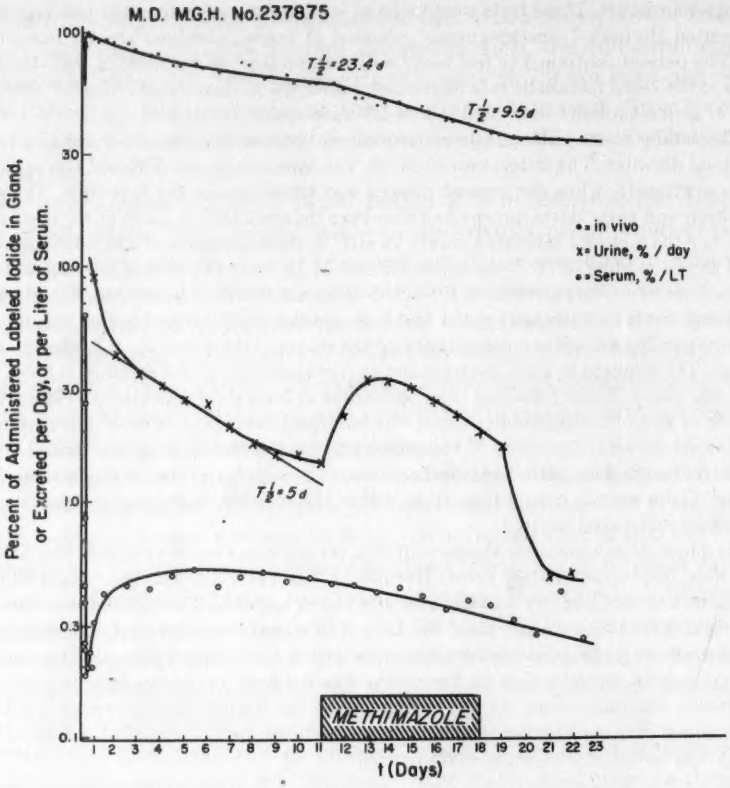

FIG. 4. *Case 4.* The fate of labeled iodine in a 25-year-old goitrous cretin as influenced by administration of methimazole. The construction of the chart is similar to that of Figure 1. Methimazole was given from the eleventh through the seventeenth day in a dose of 30 mg. every eight hours.

Labeled iodine of the blood: The concentration of labeled iodine in the blood fell rapidly during the first few minutes, then assumed an exponential slope, and after a few hours began to rise rapidly. The concentration of iodide in the iodide compartment, which was calculated from extrapolation of the iodide phase (the descending limb of the blood curve) was 1.6 per cent per liter. The iodide compartment was therefore 62.5 liters. The data do not permit a breakdown of this figure to give directly the iodide space of the gland but, assuming an extrathyroidal iodide space of 0.30 liter per kilogram, the iodide compartment of the gland was 44.5 liters or 71.2 per cent of the iodide compartment of the body. The ascending limb of the blood curve appeared after two hours, and reached a peak of 0.52 per cent per liter on the fifth day. After this, the blood concentration

declined slowly and then more rapidly and seemed to be approaching a rate of fall similar to that of the gland.

Excretion of labeled iodine: The labeled iodine in the urine was measured in half-hourly fractions for the first few hours and daily thereafter. The iodide clearance calculated for four periods after the first half-hour was 30.5 cc. per minute, or 2.8 per cent of the iodide compartment per hour. The curve of labeled iodide excretion was linear for seven days after the initial period of rapid excretion. This part of the curve had a rate constant of 13.9 per cent per hour ($T\frac{1}{2} = 5.0$ days), and intersected the axis at 5.0 per cent. When methimazole was begun, there was a threefold rise in the labeled iodide excretion rate, and a similar rise in the daily excretion of I^{127}. Thereafter the excretion rate of labeled iodide fell at a rate with a half-time of 5.3 days until methimazole was discontinued, whereupon the excretion rate became much slower.

Excretion of stable iodine: The mean daily excretion of I^{127} from the second through the tenth day was 152.1 μg., with a range from 126 to 231 (two-day pools). The specimen of the first day contained a huge amount of I^{127} and was thought to be contaminated. The mean daily excretion of I^{127} for the second through the seventh day of methimazole administration was 354.9 μg., with a range from 292 to 376. The mean excess I^{127} excretion during methimazole administration therefore was 202.8 μg. per day.

Gland weight and iodine content: Eight fragments of thyroid tissue from various regions of the excised gland were assayed for labeled iodine. The weighted mean concentration was 0.732 per cent per gram. The excised gland contained by direct measurement 74.7 per cent of the administered dose. Therefore, the estimated weight of the gland was 102.0 grams. Actually, the excised gland weighed 159.5 grams, but it contained numerous degenerated cystic areas filled with old blood. Since these areas were not contributing to the physiologic processes of the gland, the weight of metabolizing tissue could be taken to be 102.0 grams.

Four of the fragments mentioned in the previous paragraph were homogenized, precipitated, and the precipitates washed several times. The iodine content of the precipitate and of the pooled supernatant fluid was measured; 10.2 per cent of the iodine was present in the washings. In pooled washings from four other fragments, 13 per cent of the total labeled iodine was in the supernatant fluid. The weighted mean iodine content of the four fragments of tissue was 173.8 μg. per gram. Using the weight of functioning thyroid tissue as determined in the preceding paragraph, the total iodine content of the gland was therefore 17.7 mg. The specific activities of the four fragments varied from 3.94 to 5.45 per cent per mg. The specific activity estimated from *in vivo* measurement on the day of excision was 4.65 per cent per mg.

Iodinated compounds of blood and gland: It was of particular interest

to assess the chemical nature of the iodinated components of the blood and of the thyroid gland of this patient. Blood samples were procured 7, 13, 24, 36, 48, 60 and 79 hours after the 900-μc. tracer dose of I^{131}. By paper chromatography it was readily shown that thyroxine and triiodothyronine were present in all samples of the peripheral blood. Yet, in spite of heavy labeling of the gland, it was impossible to demonstrate either thyroxine or triiodothyronine in four tissue specimens at the time of operation, although a considerable amount of iodide and abundant quantities of monoiodotyrosine and diiodotyrosine were demonstrated with ease. The method used would have detected any iodinated compound which contained more than 2 per cent of the labeled iodine of the gland. The entire remaining 130 grams of thyroid tissue were therefore homogenized, hydrolyzed, and analyzed for thyroxine by the method of Gross and Pitt-Rivers (11); it was possible to demonstrate only a trace.

DISCUSSION

Case 1

The findings confirmed the previous hypothesis (3) that the thyroid of this patient concentrated iodide from the blood, but lacked the cellular metabolic process for converting iodide to an organically bound form. Presumably the enzymatic oxidation of iodide to iodine did not occur, although the observed events could also be attributed to absence of other enzymatic processes concerned in the organic binding of iodine. The dynamic behavior of the labeled iodide given to the patient was reasonably predictable on the basis of the hypothesis. Thus, the release rate of the labeled iodine of the gland was predictable from the rate of renal clearance of iodide and the volume of the iodide compartment. The similarity of gland and urine specific activities confirmed the free exchange of the iodide of blood with that of the gland. The cellular hyperplasia observed in the pathologic specimen was compatible with a large compartment of thyroid iodide.

Final confirmation of the hypothesis regarding the metabolic defect was obtained by direct analysis of the gland. No iodinated compounds could be detected by methods which easily demonstrate these compounds in normal or hyperplastic glands. Direct chemical analysis of the gland showed that its iodide content was very low, and confirmed the content predicted from the rates of labeled iodide disposal and iodide excretion.

Case 2

The disposal of labeled iodide was consistent with the hypothesis that methimazole completely blocked the conversion of trapped iodide to or-

ganically bound iodine. The rate of loss of labeled iodine from the gland was predictable from the renal clearance rate of iodide. The iodide compartment of the gland was small. The parallelism between the dynamics of iodine metabolism in this patient and in the previous one is confirmatory of the concept that no iodide was being utilized by the gland of either patient in the formation of hormone, and that in both the disposal of iodide was largely governed by renal clearance.

Case 3

The findings in the patient with Graves' disease were similar, excepting that methimazole failed to produce a complete block to hormone synthesis. A small fraction of the trapped iodide was converted to organically bound components of the gland. The iodide compartment of the gland was much larger than that of Case 2. The extrathyroidal iodide compartment was normal in size. The rate of disappearance of the labeled iodide from the iodide compartment of the gland appeared to be governed largely by the renal clearance rate and the magnitude of the iodide compartment.

Case 4

Study of the *in vivo*, blood, and urine curves and the other analytical findings from the second goitrous cretin led to certain difficulties of interpretation, as follows:

a. The release of labeled iodine during the methimazole period was exceedingly rapid; 7.3 per cent of the gland content of labeled iodine left the gland each day. If labeling of the iodine of the gland had been uniform, there should have appeared in the urine more than a milligram of I^{127} daily. Actually, there was an increase of only 0.2 milligram daily after methimazole was begun.

b. As a corollary, the specific activity of the urine was unusually high. At the peak excretion during methimazole administration the specific activity was 17 per cent per milligram, whereas that of the gland as a whole at that time was only 2.9 per cent per milligram.

c. A huge amount of unlabeled iodine left the thyroid gland daily. This was estimated from the equilibrium relation (13):

$$H = \frac{EU}{1 - U} \qquad \text{(II)}$$

where H is the quantity leaving the gland, E the daily excretion of I^{127}, and U the fractional uptake. When U is so nearly 1 (*i.e.*, 100 per cent), as in this case, the calculation is very sensitive to small changes in U. The calculated value for H was at least a milligram. The normal amount of hormonal iodine secreted per day lies between 50 and 150 micrograms.

d. The finding of triiodothyronine and thyroxine in the blood, but virtually only monoiodotyrosine and diiodotyrosine in the gland was unusual and anomalous

The usual model of thyroid function comprises a chain of linked reactions ultimately leading to the release of hormone. No such simple catenary model can explain the facts observed in this patient. If the quantity of iodine leaving the gland had all been in the form of thyroid hormone the patient would have been thyrotoxic. Although hormone was produced, a large proportion of the iodine released from the gland was not in the form of hormone. Further, the high specific activity of iodine in the urine during the methimazole block indicated that this iodine was derived from a compartment of the gland which had a specific activity which was much higher than that of the gland as a whole.

An alternative model designed to explain the data is shown in Figure 5. Iodine in the gland is considered to occupy two compartments. The first compartment is assumed to be small in volume, and to have a high uptake

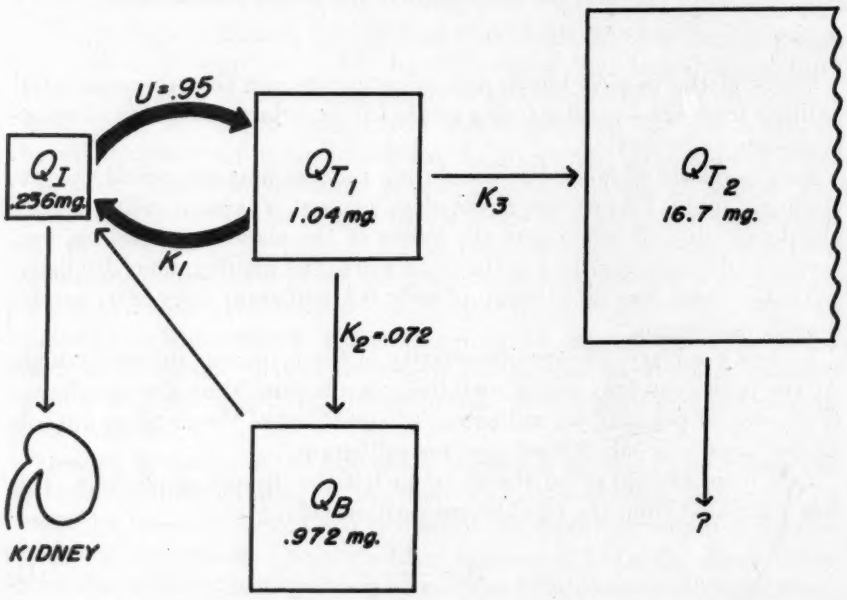

FIG. 5. A model to illustrate the dynamic state of iodine metabolism in *Case 4*. Q_I is the iodide compartment of the thyroid and extrathyroidal tissue; Q_{T1} and Q_{T2} are the postulated two compartments of the gland; Q_B is the extrathyroidal hormone compartment; K_1, K_2, and K_3 are rate constants. U is the fractional uptake. The heavy arrows represent the large amount of iodine of the first compartment which is turned over. For full discussion, see text.

and turnover. From this compartment iodine is transferred 1) into the extrathyroidal hormone compartment, 2) into a much larger compartment within the gland, and 3) into the iodide compartment of the body. The second compartment is assumed to be large. Its iodine is slowly derived from the first compartment, and its hormone is slowly released into the blood. It follows that the events which were observed early after the administration of labeled iodide would be largely governed by the activity of the first compartment. Only later would the second compartment have an appreciable effect on the urine curve or the quantity of labeled hormone in the peripheral blood. As a first approximation, therefore, it is assumed that the early portion of the urine curve mirrored the first compartment. Values for compartment sizes and rate constants of transfer based on these hypotheses and assumptions can be calculated.

The quantity of iodine in the first compartment, Q_{T_1}, is estimated from the following relation:

$$Q_{T_1}K' = H(1 - U) \qquad \text{(III)}$$

where K′ is the net rate of loss of iodine from the first compartment. Loss of iodine from the first to the second compartment has been neglected. Substituting from equation II:

$$Q_{T_1} = \frac{EU}{K'} \qquad \text{(IV)}$$

Substituting 0.95 for U, measured values for E from the second to the ninth days, and the K′ obtained from the slope of the urine curve, Q_{T_1} is calculated to be 1.04 mg. Since the total iodine in the gland was 17.7 mg. by measurement, Q_{T_2}, the iodine of the second compartment was by difference, 16.7 mg.

The rate constant of transfer from the first compartment to Q_B, the extrathyroidal hormone compartment, can only be approximated by assigning a value for the rate of hormone synthesis. If 75 μg. of hormonal iodine is assigned, the rate constant, K_2, is .072. The size of Q_B is estimated from the protein-bound iodine concentration of the serum and an estimated volume of distribution of 30 per cent of body weight. More labeled iodine than this, however, was entering the peripheral blood (discussed subsequently).

The size of the iodide compartment of the body may be estimated from the renal clearance rate of labeled iodide and the assumption that the specific activity of the urine faithfully reflects the specific activity of the iodide of the blood, thus:

$$\frac{E^*}{E} = \frac{Q_I^*}{Q_I} \qquad \text{(V)}$$

where E* and E are the daily urinary excretion rates of labeled and unlabeled iodide, respectively, and Q_I^* and Q_I are the quantities of labeled and unlabeled iodide in the iodide compartment. Since E^*/Q_I^* is the clearance rate divided by the volume of distribution of iodide, and E is measured, Q_I can be calculated. This proved to be 2.36 mg. Obviously, this value would fluctuate widely, depending upon the intake of iodide.

The specific activity of the urine during the methimazole block can be predicted from the dimensions of the first thyroid compartment and the assumptions that have been made. Extrapolation of the decay curve of the first compartment from zero time (when 95 per cent of the administered dose was in the gland) to the time of maximal urinary excretion during the block indicates that 16.8 per cent remained in the first compartment at this time. The quantity of iodine in this compartment was calculated to be 1.04 mg. The predicted specific activity of iodide in the urine, based on the hypothesis that events were entirely governed by the behavior of the first compartment, was therefore $16.8/1.04 = 16.2$ per cent per mg. The observed specific activity of the excess iodide appearing in the urine at the time of maximal urinary excretion during the block was 12.0 per cent per mg. At the end of the methimazole period the predicted specific activity was 7.1, and the observed value 6.4 per cent per mg.

The data do not permit calculation of the rate constants of transfer from the first thyroid compartment to the second, or of the release constant from the second compartment. It is also not possible to calculate the rate of degradation of the hormone in the peripheral hormone compartment or the rate of release of iodide from Q_{T_1} to Q_I. The latter value can only be approximated from equation II, and was very high. The fact that commensurate amounts of iodide did not appear in the urine during methimazole administration indicates that in this very hyperplastic gland the drug failed to block completely the organic binding of iodide.

In order to test the validity of the formulation of Figure 5, the observed curves of Figure 4 were compared with those obtained from the analogue-computor analysis based on the compartment size and rate constants shown in Figure 5 (14).[3] The *in vivo* curves and the urine curves were entirely comparable. The observed blood curve, however, rose much more rapidly than did the corresponding curve from the computor. This would be expected if a significant proportion of the labeled iodine of the serum were in some relatively rapidly excreted form other than thyroxine (for example, iodide) or if large amounts of hormonal iodine were entering the blood and being disposed of more rapidly than normal. The latter possibil-

[3] The authors wish to thank Dr. Gordon L. Brownell for performing this analysis.

ity seems unlikely in view of the fact that a normal amount of desiccated thyroid has maintained the patient in the euthyroid state since operation.

One can only speculate on the biochemical nature of the two compartments and the defect of metabolism of this patient. The simplest explanation is that coupling of diiodotyrosine to yield thyroxine took place only to a limited degree, perhaps because of a partial or total deficiency of a coupling enzyme. Such thyroxine as was formed, however, was secreted rapidly, accounting for its almost complete absence in the gland despite normal concentration in the peripheral blood. The first compartment, therefore, represents the iodinated organic moiety of the gland which was in rapid flux 1) to form hormone, 2) to furnish iodide to the blood after deiodination, and 3) to furnish iodine to the second thyroid compartment. The second compartment comprised the large amounts of monoiodotyrosine and diiodotyrosine stored by the gland.

SUMMARY

1. The fate of labeled iodine has been examined in 2 goitrous cretins and in 2 patients taking methimazole. The thyroid glands of the cretins were available for further study.

2. Disposal of labeled iodide by the first goitrous cretin was consistent with the hypothesis that the gland accumulated iodide but failed to convert it to an organic form. The declines in labeled iodide of gland and blood were governed by the renal clearances of iodide. The absence of organic iodine in the gland was confirmed chemically. The total quantity of iodine in the gland was very small.

3. When taking methimazole, both a subject with a normal thyroid gland and a patient with Graves' disease disposed of iodide in a manner similar to that in the first goitrous cretin, with the exception that the iodide compartment of the gland of the Graves' patient was larger, and the methimazole block was incomplete. Renal excretion of iodide governed the metabolic fate of iodide in the subject with the normal gland, and nearly so in the patient with Graves' disease.

4. The second cretin had a compensatory goiter, a normal basal metabolic rate, and a high uptake of I^{131}. The disposal of labeled iodide was entirely different from that in the 3 other patients. Direct analysis demonstrated labeled thyroxine and triiodothyronine in the blood, but only a trace of thyroxine in the gland, although labeled monoiodotyrosine and diiodotyrosine were found in the gland in abundance. The explanation proposed is that organic binding of iodine by the gland was enhanced, but enzymatically controlled hormone synthesis from iodinated tyrosine was impaired.

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EFFECT OF LONG-TERM ADMINISTRATION OF DESICCATED THYROID ON SERUM LIPOPROTEIN AND CHOLESTEROL LEVELS*†

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IN A previous publication (1) it was shown that the concentration of certain of the low-density serum lipoproteins, *i.e.*, the Standard S_f0–12 and Standard S_f12–20 lipoprotein classes, can be significantly lowered by the administration of desiccated thyroid. In those studies the effects were evaluated of 10 grains of USP thyroid daily administered over a period of nine to ten weeks and of 3 grains of thyroid daily over a period of three weeks. On the 10-grain regimen, the 11 patients studied showed significant and maintained reduction in the concentration of Standard S_f0–12 and Standard S_f12–20 lipoprotein classes during the nine to ten weeks of thyroid administration. It became of interest to know the minimum dosage of thyroid required to produce and maintain lipoprotein depression. The early experience with 3 grains of USP thyroid daily had shown that an acute response, at least, could be obtained; therefore, the present studies were initiated at this dosage level.

MATERIALS AND METHODS

The subjects chosen for the study were 30 male and 30 female patients with schizophrenia, who were otherwise clinically healthy. The range of ages for the males was 20–59 years, with a mean of 40.3 years; the range for the females was 20–59 years, with a mean of 39.6 years. All patients

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were institutionalized, which made it possible to control dietary factors and to assure that all medications were actually taken daily in the indicated dosage. No patients were lost to the study as a result of intolerance to thyroid. There were some losses due to two other causes: 1) patients leaving the institution, and 2) refusal of patients to permit continued blood sampling. Each patient had two lipoprotein studies, four days apart, before the beginning of thyroid administration. While receiving thyroid, each patient had a serum lipoprotein determination at intervals of three weeks. The method of ultracentrifugal analysis for the low-density serum lipoproteins[1] has been described previously (2, 3). Serum cholesterol determinations were performed by the procedure of Colman (4).

RESULTS

Data were collected on 26 males and 24 females who completed a period of thirty-three weeks of administration of 3 grains of desiccated USP thyroid daily. Since no significant differences in response to thyroid were demonstrable between males and females, the groups were combined to yield a total series of 50 patients. Mean levels of Standard S_f0–12, Standard S_f12–20, Standard S_f20–100, and Standard S_f100–400 lipoproteins, serum cholesterol, and body weight throughout the course of the 33-week period of thyroid administration are given in Table 1.

By applying significance tests to the data presented in Table 1, it was found that both mean Standard S_f0–12 and mean Standard S_f12–20 lipoproteins showed marked reductions three weeks after the initiation of thyroid therapy in a dosage of 3 grains daily. Thereafter there was a progressive rise in mean levels of the Standard S_f0–12 until the twenty-fourth week, after which the levels were not significantly different from the pre-thyroid level. The mean Standard S_f12–20 lipoprotein level did show a minimal trend upward in the course of the next thirty weeks. However, at thirty-three weeks, when the Standard S_f0–12 lipoproteins were essentially at pre-thyroid levels, the concentration of Standard S_f12–20 lipoproteins was still markedly lower than before thyroid thirapy. No significant alteration in the concentration of mean Standard S_f20–100 or mean Standard S_f100–400 lipoproteins could be demonstrated throughout the 33-week

[1] A convenient method for naming and classifying the lipoproteins is based upon the rate of flotation, under defined conditions, of the various lipoproteins in an intense centrifugal field. The unit is the "Svedberg of flotation" or the S_f. Thus, a single lipoprotein may be referred to by its flotation rate as an S_f4 or S_f10 lipoprotein. If a series of lipoproteins is under consideration, it may be referred to as the sum of the concentrations of all the lipoproteins between arbitrary flotation-rate limits; for example, the concentration of S_f12–20 lipoproteins is the sum of the concentrations of all lipoproteins floating with rates between S_f12 and S_f20.

TABLE 1. STUDY OF 50 SUBJECTS (26 MALES AND 24 FEMALES) RECEIVING 3 GRAINS OF THYROID FOR THIRTY-THREE WEEKS

Weeks on 3 grains of thyroid	Mean standard S_f0–12 (mg./100 ml.)	Mean standard S_f12–20 (mg./100 ml.)	Mean standard S_f20–100 (mg./100 ml.)	Mean standard S_f100–400 (mg./100 ml.)	Mean serum cholesterol (mg./100 ml.)	Mean weight (pounds)
0*	356.1	75.5	78.9	36.3	215.6	142.4
3	253.3	43.7	77.3	29.9	156.7	—
6	279.5	43.8	87.4	35.4	174.3	136.9
9	288.4	44.1	86.2	37.6	179.9	137.0
12	295.6	46.7	75.9	27.6	181.3	136.2
15	306.1	43.7	82.6	25.6	187.9	136.3
18	—†	—	—	—	—	136.6
21	338.2	42.0	82.8	32.0	192.8	136.4
24	375.2	49.8	78.0	26.6	213.0	136.7
27	354.8	57.0	81.9	34.9	210.3	138.2
30	—	—	—	—	—	—
33	343.6	52.4	80.6	34.4	208.6	—

* Sample "0" represents the mean of two samples from each individual, four days apart, before the administration of thyroid.

† Data not obtained for sampling intervals designated in this manner.

period of thyroid administration. Serum cholesterol trends paralleled closely those of the Standard S_f0–12 lipoproteins. Body weights showed a mean drop of approximately 5 pounds during the first six weeks of administration of thyroid, after which the mean weight remained essentially stable for the subsequent twenty-seven weeks of thyroid therapy.

The general pattern of response to 3 grains of thyroid paralleled our previous experience with a dosage of 10 grains, in that the most significant effects were observed upon the Standard S_f0–12 and Standard S_f12–20 lipoproteins. In neither experiment was there a definitely significant (1 per cent level) change in the Standard S_f20–100 or Standard S_f100–400 lipoprotein levels. One interesting difference between the 10-grain study and the 3-grain study was that, in the former the Standard S_f0–12 lipoprotein level showed no significant trend upward during the 9-week period of study, whereas in the latter there was a trend upward in the Standard S_f0–12 lipoprotein level after the third week.

An evaluation was made of the magnitude of the lowering of lipoprotein concentration at its maximum (three weeks of therapy with 3 grains of thyroid), in relation to the initial concentration. For this purpose the two classes showing a response to thyroid were summed as the Standard S_f0–20 lipoprotein class (Standard S_f0–20 = Standard S_f0–12 + Standard

TABLE 2. RELATIONSHIP OF MAXIMAL LOWERING* OF STANDARD S_f0–20 LIPOPROTEINS TO PRE-THYROID LEVEL OF STANDARD S_f0–20 LIPOPROTEINS

Range of prethyroid Standard S_f0–20 lipoprotein conc. (mg./100 ml.)	No. of cases	Mean pre-thyroid Standard S_f0–20 lipoprotein conc. (mg./100 ml.)	Mean Standard S_f0–20 lipoprotein at 3 wks. on 3 gr. thyroid daily (mg./100 ml.)	Mean change in Standard S_f0–20 lipoprotein concentration (mg./100 ml.)
Over 500	15	556	366	190
400–499	22	453	310	143
300–399	12	363	250	113
Under 300	9	248	207	31

* In these observations, maximal lowering in Standard S_f0–20 lipoprotein concentration occurred at three weeks after the institution of 3 grains of thyroid daily.

S_f12–20). From the data presented in Table 2 it is evident that the higher the initial concentration of Standard S_f0–20 lipoproteins, the greater was the response to a given dosage (3 grains daily in this case) of thyroid, when response was based upon the absolute magnitude of concentration change.

Data were also obtained on a random segment of the over-all group just described when the dosage of thyroid was increased to 4 grains daily after a period of thirty-three weeks on the 3-grain schedule. These findings, based upon 27 cases, are presented in Table 3. It may be seen that the Standard S_f0–12 lipoproteins, which had returned essentially to the pre-thyroid level after thirty-three weeks of thyroid therapy in a dosage of 3 grains per day, showed a progressive drop in level after three and six weeks on the increased dosage of 4 grains per day. It is worthy of note that three weeks after starting 3 grains per day the level of Standard S_f0–12 had reached the lowest point observed for the entire thirty-three weeks on that dosage, and that the six-week observation showed a distinct rise over the three-week level. In the 4-grain period, the six-week point showed, in contrast, a greater decline in Standard S_f0–12 lipoprotein concentration than was evident at three weeks. Whereas the mean values suggested a possible downward trend in Standard S_f0–12 lipoproteins between the twenty-fourth and thirty-third weeks, even on a dosage of 3 grains per day, a consideration of individual cases indicated that approximately half the Standard S_f0–12 values had either reached a plateau or were rising at the time when the dosage was increased to 4 grains per day.

Under the circumstances of this study, the administration of 4 grains of thyroid daily following thirty-three weeks on 3 grains daily did not cause further lowering of the Standard S_f12–20 level, nor did it cause sig-

TABLE 3. STUDY OF 27 SUBJECTS RECEIVING 3 GRAINS OF THYROID FOR THIRTY-THREE WEEKS AND 4 GRAINS OF THYROID FOR SIX WEEKS

Weeks on 3 grains of thyroid	Dosage of thyroid (gr./day)	Mean Standard S_f0–12 (mg./100 ml.)	Mean Standard S_f12–20 (mg./100 ml.)	Mean Standard S_f20–100 (mg./100 ml.)	Mean Standard S_f100–400 (mg./100 ml.)
0*	0	358.8	73.1	81.8	38.0
3	3	242.9	42.5	84.0	35.0
6	3	281.4	42.6	91.0	38.4
9	3	288.7	47.4	96.5	45.9
12	3	302.0	48.5	76.2	27.3
15	3	307.4	42.1	87.6	29.5
18	3	—†	—	—	—
21	3	338.4	40.6	84.7	33.9
24	3	374.6	33.9	81.2	26.7
27	3	348.9	54.1	84.5	36.0
30	3	—	—	—	—
33	3	338.3	50.6	85.7	37.4
36	4	324.3	40.9	71.7	28.1
39	4	303.5	44.0	77.7	28.3

*† See footnotes to Table 1.

nificant lowering of Standard S_f20–100 or Standard S_f100–400 lipoprotein levels.

Further study of the long-term aspects of the 4-grain/day schedule is being made.

DISCUSSION

The daily administration of 3 grains of thyroid causes a lowering of Standard S_f0–12 and Standard S_f12–20 lipoprotein levels without significant alteration of the Standard S_f20–100 and Standard S_f100–400 lipoprotein levels. The effect upon the concentration of Standard S_f0–12 lipoproteins is maximal in three weeks and subsequently decreases until at about twenty-four weeks the concentration has, on the average, returned essentially to the pre-thyroid level in spite of continuation of the daily 3-grain schedule. The basis for this "escape" phenomenon is not understood at this time. One plausible explanation involves the well-established thyroid-thyrotropic hormone interrelationship (5). The administration of exogenous thyroid presents the pituitary gland with a stimulus for decreased thyrotropin output. A decreased thyrotropin output causes lowered production and delivery of thyroid hormone. This decreased output of thyroid hormone results in a decrease of circulating thyroid hormone to a level

below that which would be anticipated from a combination of exogenous thyroid administration plus an *unaltered* endogenous supply from the thyroid gland. Considering such factors as the rate of decay of existing blood thyroid hormone and variation in the degree of suppression of the thyroid gland by decreased thyrotropin supply, it is not unreasonable to expect that the net effect of administration of exogenous thyroid may be (at least temporarily): 1) an increase, 2) no change, or even 3) a decrease in the concentration of circulating thyroid hormone. If, in the serum, the control

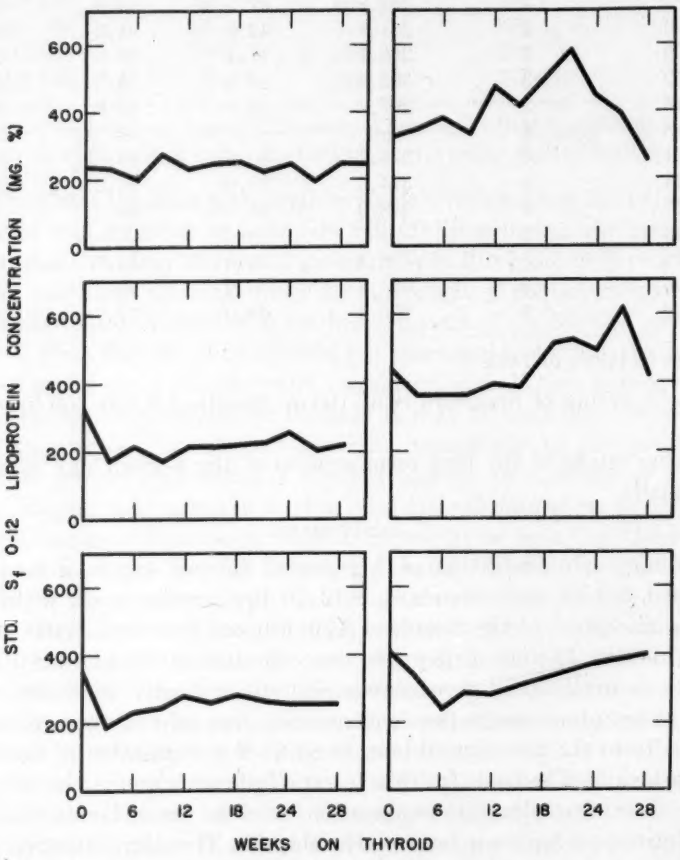

FIG. 1. Typical response curves of serum lipoproteins of the Standard S_f0-12 class while the 6 euthyroid subjects were taking desiccated thyroid. The first point plotted on each curve is the mean of two Standard S_f0-12 measurements. All other measurements were made while the subjects were maintained on a regimen of 3 grains of thyroid daily.

of the level of Standard S_f0–12 lipoproteins is related to the level of circulating thyroid hormone, the net effect of administration of exogenous thyroid may be: 1) to lower, 2) leave unaltered, or 3) raise the serum Standard S_f0–12 level. All three types of response and combinations thereof were observed in the course of the thirty-three weeks of administration of 3 grains of thyroid per day. In Figure 1 are shown representative response curves. Another possibility for partial explanation of the various response curves is that the inactivation or destruction of thyroid hormone is variably affected by the administration of desiccated thyroid. If our first explanation outlines the primary mechanism for the observed changes, it would be anticipated that with a sufficient increase in the dose of exogenous thyroid, a point should be reached where, even with complete shutdown of hormone output by the thyroid gland, the circulating hormone level would be higher than that in the pre-thyroid period. Under these conditions presumably there would be a sustained lowering of the Standard S_f0–12 lipoprotein level. In the previous study with 10 grains per day, the prolonged drop in Standard S_f0–12 level over a period of nine to ten weeks suggests such a sustained decrease. The present study, involving progressive increases in exogenous thyroid above 3 grains per day, may allow more extensive testing of this hypothesis, and also determination of the dose required to produce such sustained lipoprotein lowering, assuming the later is possible.

SUMMARY

1. In a series of 50 schizophrenic patients receiving a daily dosage of 3 grains of desiccated thyroid, the concentrations of the mean Standard S_f0–12 and mean Standard S_f12–20 serum lipoproteins were significantly reduced within a period of three weeks, but by twenty-four weeks the Standard S_f0–12 concentration had returned to the pre-thyroid level.

2. The magnitude of the drop in the Standard S_f0–12 concentration was directly related to the pre-thyroid value.

Acknowledgment

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THE MECHANISM OF INDUCTION AND THE CHARACTERISTICS OF PITUITARY TUMORS INDUCED BY THYROIDECTOMY*†

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DESTRUCTION of the thyroid by radioiodine gives rise to pituitary tumors in all strains of mice thus far tested (1–4). Sustained deficiency of thyroid hormone (TH) leads to hyperplasia of pituitary cells secreting thyroid-stimulating hormone (TSH), followed by the development of tumors that are responsive to the restraining force of TH. Transplantation of pituitary tumors induced by thyroidectomy succeeds only in mice with thyroids which have been destroyed by I^{131} or removed surgically. These tumors grow also in hosts subjected to subtotal thyroidectomy, but after a longer latent period and at a slower rate. The relation of proliferation of thyrotropic tumor cells to thyroid function is inverse and quantitative (5). In the course of continued subpassages in mice these dependents tumors give rise to autonomous variants. The autonomous tumors grow better in normal than in athyroid hosts.

Part of the work surveyed here has already been reported (3–7), and part will be described later (8, 9). This report is a summary of all relevant observations which have led us to the conclusion that sustained deficiency of thyroid hormone gives rise to thyrotropic pituitary tumors, whether the thyroid deficiency be brought about by ionizing radiation or by other agents.

NOMENCLATURE

The changes in pituitary structure after administration of I^{131} are tabulated as follows: a) thyroidectomy changes, characterized by large chromophobe cells scattered

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throughout the gland (10); b) microtumors (Fig. 6), characterized by minute nodules of large chromophobes (10) or amphophils (11); and c) gross tumors (Fig. 3). Primary tumors are those originally induced by thyroid depression; dependent tumors are grafted growths which grow only in hypothyroid hosts, and autonomous tumors are those which grow in both normal and hypothyroid hosts (2). By radiothyroidectomy is meant the total or nearly total destruction of the thyroid by I^{131}. The tumors induced by thyroidectomy will be referred to as thyrotropic pituitary tumors (or thyrotropic tumors), even though the possibility has not been excluded that they secrete traces of gonadotropins (6). Radiation-induced pituitary tumors secreting ACTH (12, 13) will be referred to as adrenotropic tumors.

MATERIAL AND METHODS

For information concerning animals used, diet, administration of I^{131}, and isotopic and other procedures, the reader is referred to the original articles (2, 5). The technic of surgical thyroidectomy has been described elsewhere (8).

The mice detailed in the tables were of the C57BL strain. Other strains in which pituitary tumors were induced by thyroidectomy and serially transplanted were the RF, LAF_1 and RAF_1 strains. For routine transplantations tumors were removed under aseptic conditions, cut into small fragments, placed in physiologic sodium chloride solution, and injected intramuscularly in a volume of about 0.05 cc., with care that each animal should receive some tumor fragments. As a rule, batches of 6 to 10 each of normal and thyroidectomized mice were injected. Transplantation of both dependent and autonomous

TABLE 1. INCIDENCE OF PITUITARY TUMORS IN MICE KEPT ON NORMAL DIET, IN RELATION TO TIME AFTER ADMINISTRATION OF I^{131}*

Dose of I^{131}	Type of pituitary change	Males			Females		
		Days after administration of I^{131}					
		250–349	350–449	More than 450	250–349	350–449	More than 450
25 μc.	Normal	1	3	9		1	4
	Thyroidectomy changes			3		2	4
	Microtumor					2	6
	Gross tumor					1	
50 μc.	Normal			6	6		
	Thyroidectomy changes			3	3	1	
	Microtumor		1	3	4		
	Gross tumor		1	8	1	1	7

* Normal C57BL mice, approximately 3 months of age, were given the indicated amount of carrier-free I^{131}. The survivors were killed nineteen months after this treatment.

TABLE 2. EFFECT OF X-RAYS ON INDUCTION OF PITUITARY TUMORS BY I^{131} IN MICE ON LOW-IODINE DIET*

Treatment		Survival in days after I^{131}	Number of mice			
			Males	Females		
I^{131}	X-rays		No tumor	No tumor	Micro-tumor	Gross tumor
30 μc.	—	240–364		1	6	1
		365–405	6		7	2
—	500 r, total body	365–406		23		
30 μc.	500 r, total body	240–364	4	2	8	5
		365–401	5			
30 μc.	500 r, head & neck	240–364	2	1	5	6
		365–398		4	5	
30 μc.	500 r, abdom. region	264–364		3	3	
		365–402	7		5	10
30 μc.	50 r, total body	264–364		2	5	1
		365–408	8		4	7
—	—	365–432		8		

* C57BL mice, 3 months of age, were on a low-idoine diet for one month prior to administration of I^{131}. Insignificant groups with small numbers of animals were omitted from the tabulation. The irregularity of numbers in the groups was caused by loss due to intercurrent conditions.

tumors has been nearly 100 per cent successful in homologous strains, the dependent tumors only in athyroid hosts, and the autonomous tumors in both normal and athyroid hosts.

Sick animals were killed *in extremis*. Animals on which adequate postmortem examination could not be made were omitted from consideration. The microscopic technics used, the morphologic features of the tumors, and the secondary changes produced by them have been described (6). In experiments recorded in Tables 1, 2 and 3, the pituitary and thyroid regions were studied in numerous sections from each animal, and the thyroid in interrupted serial sections whenever deemed necessary.

The "low-iodine diet" was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. The standard laboratory diet was Purina Chow supplemented once weekly by green vegetables and hemp seed. Drinking water was unrestricted. Animals on a low-iodine diet were given distilled water only.

The factors of x-irradiation were 250 kv, 30 mà, 3 mm. aluminum filtration (beryllium window), TSD 93.7 cm., 57 r per minute skin dose rate (measured in air and corrected for temperature and barometric pressure).

TABLE 3. PITUITARY TUMOR INDUCTION BY SURGICAL THYROIDECTOMY (SERIES 1)*

Condition of pituitary	No. of mice in group	Days after operation			
		150–249	250–349	350–449	More than 450
Normal	3		1	2	
Thyroidectomy changes	2	2			
Microtumor	9	1	2	5	1
Gross tumor	11		1	2	8
Totals	*25*	*3*	*4*	*9*	*9*

* Young C57BL mice were thyroidectomized under Nembutal anesthesia. At least one parathyroid was left intact. The operative procedures and other treatment will be described elsewhere (7).

RESULTS

Factors in induction of pituitary tumors by I^{131}

Dose and sex. The smallest single dose of I^{131} which will induce pituitary tumors in mice kept on the usual laboratory diet is about 25 μc. in females and about twice as much in males (Table 1) (8). Doses of 100 μc. and 200 μc. produced pituitary tumors in all mice in which destruction of the thyroid was complete or nearly complete and which lived longer than one year.

There were great individual variations in the response of mice to threshold doses of radioiodine. Doses of 25 μc. and 50 μc. seldom destroyed the thyroid completely (Figs. 1, 2, 5), but injured it sufficiently to initiate compensatory changes in the pituitary leading to tumor formation, notably in females. There was no exception to the rule of an inverse relationship between degree of thyroid destruction and pituitary tumor development. This confirms earlier observations in which the rate of growth of dependent tumors was studied in animals with thyroids which had been destroyed by graded doses of I^{131}, the function of the thyroids having been measured by the ability of the gland to take up I^{131} (5). In both sets of experiments the individual variations were due to differences in degree of injury to the thyroid gland in different animals and, within the dose range of 25 to 400 μc., the time required for induction of pituitary tumors was closely related to the degree of thyroid destruction.

Individual variations among animals given similar doses of I^{131} were considerable. In a few instances, even a dose of 100–200 μc. failed to induce a

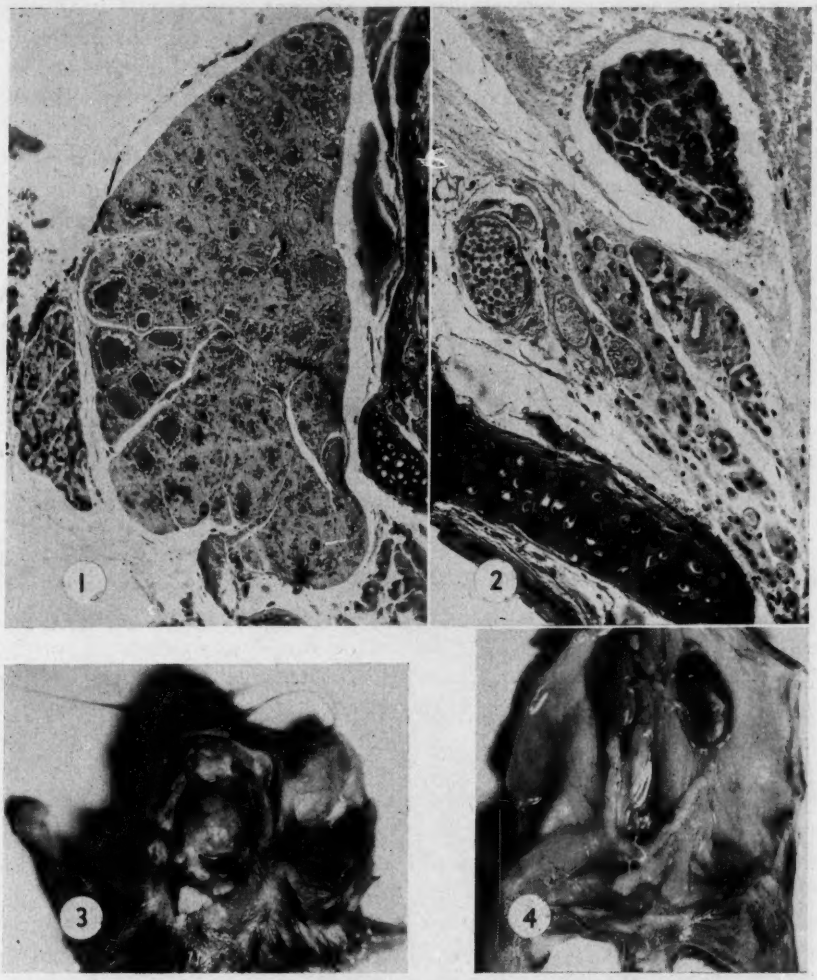

FIG. 1. The thyroid of a female mouse 543 days after administration of 25 μc. of I^{131}, showing a great increase in hyaline connective tissue and stimulated epithelial cells. Similar hyaline material is also seen in the adjacent parathyroid gland. This animal did not have a pituitary tumor. (×85)

FIG. 2. The atrophic thyroid of a female mouse 541 days after administration of 50 μc. of I^{131}. This animal had a pituitary tumor similar to that shown in Figure 3. (×150)

FIG. 3. Pituitary tumor of a mouse that had been given 30 μc. of I^{131} after four weeks of low-iodine diet and 50 r of total body x-irradiation. The animal died 330 days after the I^{131} treatment.

FIG. 4. The normal sized adrenals, ovaries and uterine horn of the animal shown in Figure 3.

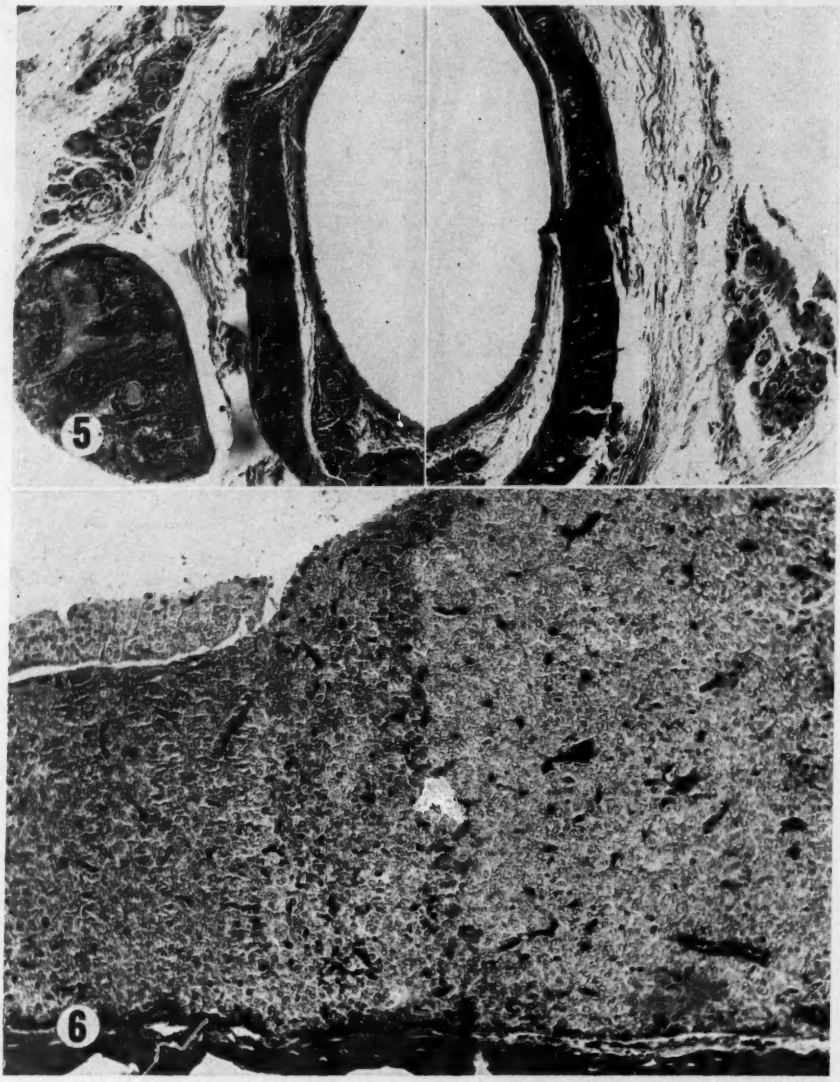

FIG. 5. Both thyroid lobes of a male mouse that had been given 50 μc. of I^{131} and died 573 days later. There are atrophic thyroid follicles on both sides and an adenoma on one side. (×80)

FIG. 6. A small tumor in a lateral lobe of the pituitary gland of the animal shown in Figure 5, with scattered tumor cells in the rest of the organ. The number of eosinophils (cells darkly stained) is greatly reduced. (×150)

pituitary tumor, and in these cases microscopic examination invariably disclosed the presence of numerous follicles with morphologic features of hypersecreting cells. In general, microtumors were evident within eight months after marked thyroid depression, and gross tumors after about one year.

In animals kept for thirty days on a commercial low-iodine diet and distilled water, 30 μc. of I^{131} greatly injured the thyroid and induced pituitary tumors in all female mice living longer than 330 days (Fig. 3), but not in males (Table 2). The tumors which developed in females replaced most of the normal pituitary; in males there were neither pituitary tumors nor distinct pretumorous changes.

Morphologic studies of the thyroids in animals of both sexes given this amount (30 μc.) of I^{131} indicated considerable injury in females and no significant damage in males. Earlier studies (5) indicated a clear-cut relationship between function (I^{131} uptake) and morphology of the thyroid after subtotal radiothyroidectomy. Since it is most unlikely that the thyroid epithelium of the female is more sensitive to the same dose of ionizing radiation than that of the male, we assume that the clear-cut difference between sexes with respect to both thyroid damage and pituitary tumor induction was due to differences in iodine uptake. Whether or not this assumption is correct is now being tested experimentally. Much is known about the greater incidence of thyroid tumors and goiters in women, but we are not aware of experimental studies showing an enhanced sensitivity of the female animal to the experimental induction of goiters and thyroid tumors.

Strains of animals and of transplantable tumors. All four strains of mice tested by us responded well to the induction of pituitary tumors, and in all three strains in which serial transplantations were attempted, tumors were induced in athyroid hosts. In fact, a gross pituitary tumor developed in every athyroid mouse which lived longer than twelve to fourteen months, depending on the strain and other factors. Those which died after six to twelve months had microtumors, and those which died earlier exhibited the characteristic changes following thyroidectomy (10).

The basis features of all transplantable tumor strains were alike. Growth of all first generation grafts was dependent on deficiency of thyroid function of the host. There were marked differences among the established transplantable tumor strains with respect to rate of growth and the rapidity at which they yielded autonomous cell variants. One slowly growing strain, carried for three years, remained dependent, whereas most others gave rise to autonomous variants soon after the second subpassage.

The hormonal spectrum of the tumors, as indicated by morphologic changes in tumor-bearing hosts, was similar in all tumor strains, the differ-

ences observed being merely quantitative. Autonomous tumors grafted in normal hosts caused a profound enlargement of the thyroid, with formation of adenomas which occasionally invaded vessels and metastasized to regional nodes but did not stimulate other endocrine organs (6). In athyroid hosts both autonomous and dependent tumors frequently caused marked stimulation of the gonads and cystic dilation of the extrahepatic biliary tracts (6). The gonadal changes were particularly conspicuous in the RF tumor strains. Both morphologic changes in tumor-bearing hosts and TSH assays by I^{131} uptake methods (9) indicated diminution of TSH production in the course of serial transplantations.

Species. Several unsuccessful experiments were made to induce thyrotropic tumors in the rat. In one of these, recently completed, 33 adequately radiothyroidectomized Wistar rats of both sexes lived for 445 to 998 days after treatment with I^{131}. Pituitary tumors occurred among them, but at no greater rate than among the 28 control rats which lived from 428 to 987 days.

There is no doubt that radiothyroidectomy fails to induce pituitary tumors in rats under conditions in which it has never failed in all strains of mice and in virtually all animals tried thus far. It is surprising, therefore that the pituitary tumors found in aged rats of two strains were attributed by Bielschowsky (14) to chronic thyroxin deficiency due to inadequate iodine content in the diet. It is possible that extrathyroidal thyroxin synthesis in rats is adequate to maintain the thyrotropes in a normal physiologic state. If this assumption be true, low-iodine diet or blocking the extrathyroidal thyroxin synthesis would lead to the development of pituitary tumors in radiothyroidectomized rats. This explanation is not entirely satisfactory, since our radiothyroidectomized rats were thyroxin-deficient, as indicated by considerable retardation in weight gain and by the presence of exophthalmos in all animals. The animals each weighed about 37 grams on the day of radiothyroidectomy; 251 days later the control males weighed an average of 304 grams, and the females 207 grams, whereas the athyroid rats weighed 157 and 119 grams respectively.

Changes in the thyroid in mice after subtotal radiothyroidectomy

There were two remarkable findings in the thyroids that had been incompletely destroyed by I^{131}: 1) failure to undergo malignant transformation, and 2) inability of residual thyroid tissue to check the induction of pituitary tumors. Sections of these thyroid remnants showed a fully disorganized structure—a few follicles with markedly stimulated epithelial cells, some undergoing adenomatoid hyperplasia, or, most frequently, a combination of these changes. Some follicles were lined by flat, and others

by large, cuboidal epithelium containing globules with staining properties of colloid similar to those seen in thyroids of normal mice given large quantities of TSH. Abnormal epithelial cells with the large hyperchromatic nuclei characteristic of malignancy were common in irradiated areas (6) yet no spontaneously spreading thyroid tumors were encountered in hundreds of radiothyroidectomized mice under our observations. Adenomas were few, and the size of the thyroid was invariably smaller than normal. Radioautographs indicated that iodine uptake in the adenomas was less than normal (5).

Since it is known that administration of TH prevents induction of pituitary tumors by thyroidectomy (12, 13) and that it controls to some extent the growth of grafted dependent tumors (7), we must conclude that the hyperplastic or newly formed cells do not function sufficiently to prevent the development of pituitary tumors.

Induction of pituitary tumors by surgical thyroidectomy (8)

Two series of experiments have been completed and a third is in progress. In the first series, 25 thyroidectomized mice were maintained until death under standard conditions. A few months after operation a dose of 7 to 9 μc. of I^{131} was administered, to determine the completeness of thyroidectomy. Pituitary tumors developed in all 9 animals of this series which lived longer than 450 days (Fig. 8), as well as in 10 of 13 mice examined post mortem between 250 and 449 days after the operation (Table 3). The time of tumor development was comparable to that in radiothyroidectomized animals. The dose used for testing thyroid function in this experiment was unnecessarily large, but was much lower than that required for induction of pituitary tumors. Furthermore, measurements of I^{131} uptake by scintillation counter gave values at background levels only; thus, it is most unlikely that this small dose of I^{131} injured the pituitary gland.

The induction of pituitary tumors in these mice is remarkable because thyroid remnants were found in 25 to 31 thyroidectomized animals (Series 1 and 2). The thyroid region in these mice was studied in interrupted serial sections. The sites of surgical thyroidectomy could be readily distinguished from those of radiothyroidectomy by a) the traumatic lesion at the operative site, b) the lack of diffuse fibrosis, sclerosing arteritis, and atypical epithelial cells (findings that are characteristic of radiothyroidectomy (6)), and also by c) the presence of large uninjured parathyroid glands and scattered, abnormally placed thyroid follicles, with occasional adenomas (Fig. 7). Thus, it is evident that the residual epithelial cells did not secrete a sufficient quantity of TH to prevent the development of pituitary tumors. In contrast, the uninjured thyroid gland has a tremendous pro-

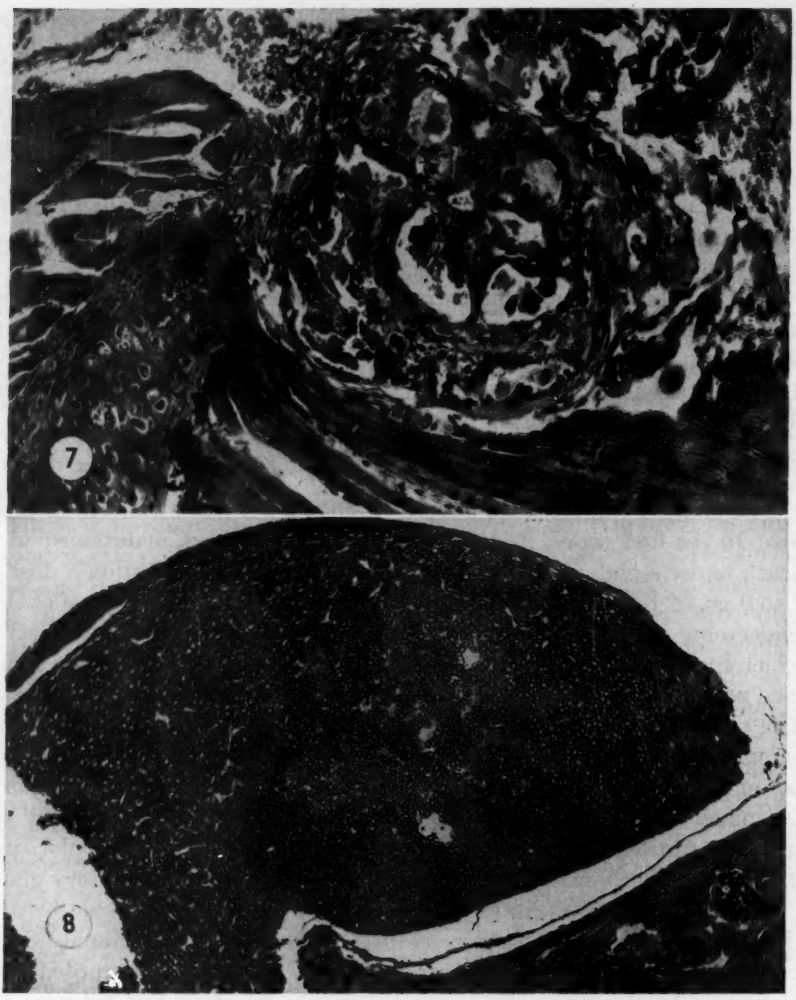

FIG. 7. Adenoma in the thyroid region in a mouse 474 days after surgical thyroidectomy. This adenoma compresses the surrounding muscle; it seems to have arisen from a thyroid fragment dislodged during the operation. The animal received a test dose of 7.3 μc. of I^{131}. (×170)

FIG. 8. The pituitary tumor of the animal shown in Figure 7. Normal pituitary cells appear only at the margin of the tumor. (×90)

liferative capacity; under the influence of a grafted TSH-secreting pituitary tumor its size may increase more than 50-fold (4, 6). It is puzzling

that the residual thyroid tissue failed to undergo an adequate compensatory hyperplasia, as there was no limiting fibrosis or stenosing arteritis and there were large quantities of circulating TSH, indicated by bioassays (9).

In the second experiment dependent tumors were grafted on 6 surgically thyroidectomized mice (not exposed to I^{131}) and 8 normal mice. The grafts grew progressively in all of the former and in none of the latter mice, indicating again that antecedent irradiation by I^{131} is not a necessary growth stimulant of thyrotropic cells. All 6 thyroidectomized mice had thyroid remnants at necropsy, performed about four months after operation. The pituitaries of these animals exhibited advanced thyroidectomy changes with tumor-like proliferation of chromophobes, and a microtumor was found in one. It seems that once the homeostatic balance between TH and TSH is tilted in the direction of TSH excess, the stimulated residual (presumably normal) thyroid cells are unable to produce sufficient quantities of TH to stop the progressive proliferation of thyrotropes.

A third experiment is in progress in which surgically thyroidectomized mice are left to age without previous administration of I^{131}. One animal of this series, dead of intercurrent disease, had a characteristic microtumor.

TABLE 4. EFFECT OF GONADECTOMY ON PITUITARY TUMOR INDUCTION BY I^{131}*

Treatment	Survival (months)	Number of mice					
		Males			Females		
		No tumor	Enlarged pituitary†	Tumor	No tumor	Enlarged pituitary†	Tumor
Gonadectomy and 300 μc. I^{131}	8–12		1	2		2	
	13–16		1	4		3	3
	more than 16		1	8			5
I^{131} only (300 μc.)	8–12					5‡	
	13–16		1	10			4
	more than 16			1			
Gonadectomy only	8–16	4			1		
	more than 16	2			2		

* The mice were castrated when 6 to 8 weeks old and I^{131} was administered one month later.

† Probably microtumor.

‡ Two of these died at 5 months.

Gonadectomy and induction of pituitary tumors

Pituitary tumors occur frequently in certain strains of mice gonadectomized when a few days old (15), and can be induced by stilbestrol in both rats and mice (2). The secretory characteristics of these tumors have not been established. A relation between gonadotropes and thyrotropes is indicated by the marked gonadal stimulation of many athyroid mice bearing large grafted, essentially thyrotropic tumors.

The possibility that gonadectomy might prevent or modify the induction of pituitary tumors by radiothyroidectomy was tested in several experiments. The results, shown in Table 4, suggest that gonadectomy does not influence the rate of tumor induction by I^{131}. In order to ascertain whether or not the character of the tumors was modified, three primary tumors induced by the combined treatment of thyroidectomy with gonadectomy were grafted on normal and on thyroidectomized hosts. Transplantations succeeded only in thyroidectomized hosts, and the changes in animals with successful grafts were indistinguishable from those caused by tumors induced by I^{131} alone.

Effect of supplementary ionizing radiation on the induction of pituitary tumors by I^{131}

Numerous observations already described argue against a major role of ionizing irradiation of the pituitary in the induction of tumors by I^{131}, as postulated by others (16). The recent report of Moore *et al.* (17) on induction of pituitary tumors by long continued administration of propylthiouracil supports this view. However, these investigators did not describe the character of pituitary tumors induced by propylthiouracil. Since the report that irradiation of the pituitary enhances tumor induction by I^{131} (13) was not based on simultaneously observed groups of animals, and the I^{131} dose used in this study was found by us to be tumorigenic in female mice without irradiation, we have reinvestigated this problem.

Seven groups of animals were studied; the experimental set-up and results are shown in Table 2. The data indicate that a dose of 30 μc. alone is tumorigenic in female mice kept on a low-iodine diet, but not in males, and that x-ray irradiation (500 r and 50 r) over the entire body or over the upper or lower half of the body alone, seems to have no noteworthy tumor-enhancing effect (Table 2). This is true particularly in males, in which the dose of 30 μc. of I^{131} was just below the threshold tumorigenic dose, and the added ionizing radiation had an excellent opportunity to manifest a co-carcinogenic effect.

The pituitary gland is known as a radioresistant organ. It was shown recently that ionizing radiation of atomic detonation (gamma rays and

neutrons) causes the development of pituitary tumors (18). An analysis of 10 of these tumors was undertaken by grafting them on normal and on variously conditioned hosts and studying the secondary changes caused by successful grafts (19, 20). By means of further serial passages, new types of transplantable pituitary tumor strains were isolated (19).

The results indicate that 3 of the 10 tumors induced by radiation secrete ACTH and no other hormone (transplantable strains O.G. 1, 2 and 6). One strain (O.G. 7) has combined thyrotropic and growth hormonal effects, and 2 others (O.G. 3 and 4) have lactogenic effects. Two (O.G. 8, 9) are still *sub judice* and transplantation of the remaining 2 tumors has thus far been unsuccessful. In contrast, all pituitary tumors induced by thyroidectomy are thyrotropic.

Thus, of the 10 assayed tumors occurring in irradiated mice, only 1 had some similarity to tumors induced by I^{131}. This strain (O.G. 7) was autonomous at the start, had a marked growth-promoting effect and, in the course of successive transplantations, rapidly lost most of its thyrotropic and growth-promoting potencies. In contrast, more than 13 primary pituitary tumors induced by radiothyroidectomy or surgical thyroidectomy proved transplantable in athyroid hosts, and none in normal hosts. It seems that the mechanism of induction and the character of pituitary tumors developing in mice after total body ionizing irradiation are different from those of pituitary tumors which arise after thyroidectomy.

Character of pituitary tumors induced by thyroidectomy

Bioassays for TSH. Numerous bioassays were performed on chicks and mice, to estimate the quantity of TSH contained in the tumors and in the blood of tumor-bearing mice. Earlier, D'Angelo (21) and Dent *et al.* (8) indicated by tadpole assays the presence of large quantities of TSH in dependent tumors. W. R. Bates (22) found by the iodine-depletion method of assay, that dependent tumors contain six to ten times more TSH per gram than anterior pituitaries of cattle, horses, or sheep, but no detectable amount of gonadotropin and prolactin. The high TSH content of these tumors was confirmed by Halmi *et al.* (23) in rat assays. Our assays in mice and chicks (9), utilizing I^{131} uptake by the thyroid, total-body retention of I^{131}, and morphologic changes caused by TSH, gave highly variable results in individual animals, but the trend was the same with the different types of materials. So far, in spite of extensive work, we are unable to give more than a crude estimation of TSH in terms of U.S.P. units, in part because of the wide individual variation in values in the same groups, notably in newborn chicks, and in part because of the much higher values obtained in the chick assays than in the mouse assays. For example, in mouse assays, the plasma of mice bearing dependent tumors indicated the presence of ~40 TSH units per cc., but there was only ~1 unit per cc.

in the chick assay. This was probably due to serologic differences between TSH preparations of different species, and we suppose that assays in heterologous species may yield false low values. Our chick assays suggest that 1 gram of the mouse pituitary contains about 1 TSH unit, and primary tumors contain at least five to ten times more TSH than the normal mouse pituitary. The grafted dependent pituitary tumors contain somewhat less hormone than the primary tumors, and autonomous tumors much less than the primary tumors. The blood hormonal levels parallel the tumor hormonal levels. In dependent-tumor-bearing mice, 0.02 to 0.05 cc. of plasma never failed to give a positive assay reaction by any method tried, including the least sensitive mouse assay.

The findings suggest a gradual drop in hormonal concentrations in the course of successive transplantations and the assumption that even the dependent tumors are composed of somewhat altered cells. It is desirable to pursue this problem further. Precise hormonal assays concomitant with cytologic examinations, performed in the course of successive transplantations of the same strain, may yield information concerning the character of these pituitary tumors and indicate whether the change in the tumor cells is gradual or stepwise.

Changes in tumor-bearing hosts. In attempts to detect the secretory properties of a pituitary tumor, it is current practice to search for a stimulated target organ. In the case of primary dependent thyrotropic tumors (Figs. 3 and 4), the thyroid is either absent or atrophic. Depression of its function is the prime inciter of tumor formation. No organ is markedly stimulated (6). In the case of primary adrenotropic tumors, atrophy of the adrenal is the characteristic finding (19). Indeed hypofunction of a target organ and its inability to undergo compensatory hyperplasia or hypertrophy are the dynamic factors in the induction of dependent pituitary tumors.

The situation is the reverse with autonomous thyrotropic tumors. Such tumors, growing in normal hosts, cause a tremendous enlargement of the thyroid, the appearance of the epithelium clearly indicating stimulation by TSH; there is no distinct morphologic change in any other endocrine organ. Similarly, autonomous adrenotropic tumors (Strains 1, 2 and 6) grafted on normal hosts cause hypertrophy of the adrenal, but no distinct change in other endocrines.

Thus, if one attempts to judge the functional attributes of primary pituitary tumors from morphologic alterations in the hosts bearing them, one encounters varied situations. With dependent (responsive) tumors an atrophy, but with autonomous (nonresponsive) tumors a stimulation of the target organ is to be expected.

The character of the pituitary tumor induced by propylthiouracil

(Moore *et al.* (17)), as mentioned before, has thus far not been ascertained. It is noteworthy that the changes in the thyroid gland and the behavior of this organ are similar in mice bearing autonomous thyrotropic tumors on the one hand and in mice treated with thiouracil or related substances on the other. The thyroids are at first uniformly enlarged, the epithelial cells exhibiting the usual features of excessive stimulation. Later, adenomas develop, sometimes metastasizing to draining lymph nodes and veins (6). When grafted in normal hosts, these thyroids perish or remain barely detectable, whereas in hosts carrying grafted pituitary tumors they proliferate wherever they are grafted. When grafted intravenously in athyroid hosts, they develop numerous pulmonary "metastases," the postmortem findings suggesting thyrotoxicosis (6).

There are two puzzling phenomena in athyroid hosts bearing thyrotropic tumors: 1) marked gonadal stimulation, and 2) cystic dilation of the extrahepatic biliary tract (6). The transplantation studies of Anderson (24) in hypophysectomized hosts suggest that these thyrotropic tumor cells secrete traces of gonadotropin in quantities below the sensitivity of the assay technics.

Histologic identification of thyrotropes. The staining reactions of the thyrotropic tumor cells, whether designated as chromophobes, degranulated basophils (10) or amphophils (11), do not conform with the current view that thyrotropes are basophils which give the Schiff periodic-acid and the aldehyde-fuchsin reactions (10). These reactions are negative or faintly positive with our thyrotropic tumor cells, the kind of fixative used altering somewhat their staining properties. The supposition that these reactions are negative because the thyrotropes discharge their hormone in hypothyroid hosts is contradicted by the observation that the staining properties of autonomous tumor cells are similar in both hyperthyroid and athyroid hosts, and the presence of large quantities of TSH in both autonomous and dependent tumors is indicated by bioassays.

SUMMARY AND CONCLUSIONS

Pituitary tumors induced by radiothyroidectomy are composed of thyrotropes which proliferate in response to a sustained deficiency of thyroid hormone. Observations are described, indicating that irradiation of the pituitary plays no significant role, if any, in the induction of these tumors. Pituitary tumors induced by total-body irradiation have a different hormonal spectrum.

Complete destruction or removal of the thyroid is not essential for pituitary tumorigenesis; subtotal surgical thyroidectomy will likewise induce such tumors. Residual thyroid tissue, although undergoing some regeneration, is apparently incapable of secreting enough thyroid hormone to main-

tain the physiologic balance between the thyroid and the pituitary. Whereas the normal thyroid undergoes tremendous hyperplasia when stimulated by autonomous thyrotropic tumors, the thyroid damaged by ionizing irradiation and the thyroid remnant after nearly complete surgical thyroidectomy fail to undergo such compensatory changes.

Primary thyrotropic pituitary tumors and first-generation tumor grafts are dependent on deficiency of thyroid hormone; administration of the latter will check their growth. When proliferation of pituitary tumor cells is sustained through subpassages in hypothyroid hosts, autonomous variants arise which are not controlled by thyroid hormone.

Formation of adenomas in the thyroid, with invasion of blood vessels and metastasis to lymph nodes, is common in thyroids of mice that have been stimulated by the TSH of autonomous thyrotropic tumors; but these changes are merely dependent growth disturbances, not sustained in normal hosts, as are the metastasizing thyroid adenomas induced by thiouracil.

The availability of dependent and autonomous tumors of both thyroid and pituitary, the ease of their induction, and the possibility of measuring their functions quantitatively provide an excellent basis for the study of thyroid-pituitary interrelationships and the problem of tumorigenesis in these organs.

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BIOLOGIC EFFECTS OF TRIIODOTHYRONINE IN HUMAN SUBJECTS*†

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THE present report is virtually an extension of the observations on triiodothyronine by McConahey *et al.* (1) and Lerman (2), presented at the meeting of the American Goiter Association in May 1953.

NORMAL HUMAN SUBJECTS

The remarkable potency of triiodothyronine[1] when given by mouth to normal subjects, shown by its ability to reduce the thyroidal uptake of a second tracer dose of radioactive iodine (I^{131}) as compared with the first has already been reported (3). This phenomenon is well demonstrated in Figure 1, in which it is noted that an oral daily dose of .0088 milligram of triiodothyronine for seven days caused a 75 per cent reduction in the uptake of the second tracer dose, as compared with the first tracer dose, in the same person. In this short space of time, and with this minute dose of triiodothyronine, there was a significant reduction in the concentration of protein-bound iodine (PBI) of blood drawn at the end of the seven days of therapy. An oral daily dose of triiodothyronine half this size, namely 0.0044 milligram, did not reduce the thyroidal I^{131} uptake of the second tracer dose. Approximately fifteen times the 0.0088-mg. dose, namely 0.140 milligram, did not produce any greater depression of the I^{131} uptake of the second tracer.

ANIMALS

This phenomenon was applied to animal laboratory work on the bioassay of TSH for the purpose of producing a physiologic TSH hypophysec-

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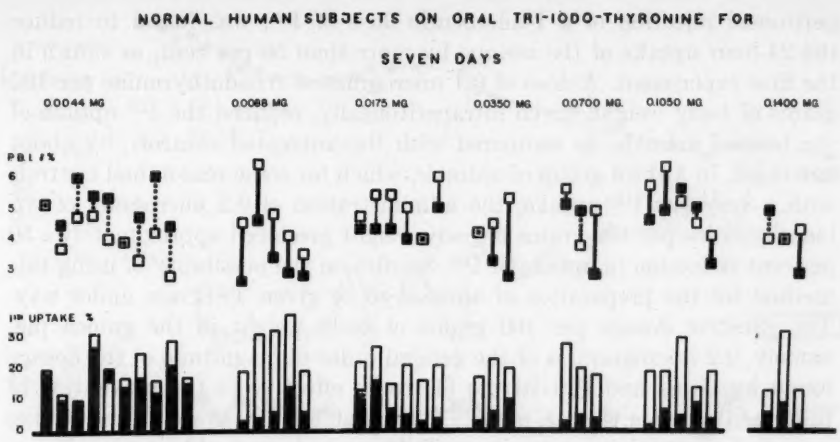

FIG. 1. Serum protein-bound iodine (PBI) and thyroidal 24-hour I^{131} uptake per centage values before (white squares and columns) and during (black squares and columns) oral triiodothyronine medication in normal human subjects. Blood was drawn for the PBI determination, and the tracer dose of isotope was administered within a few hours after the subject took the sixth daily dose of medication.

tomy. Data on 3 groups of experimental animals are shown in Figure 2. In the first group triiodothyronine given in a dose of 1 microgram per 100 grams of body weight intraperitoneally for five days before the intra-

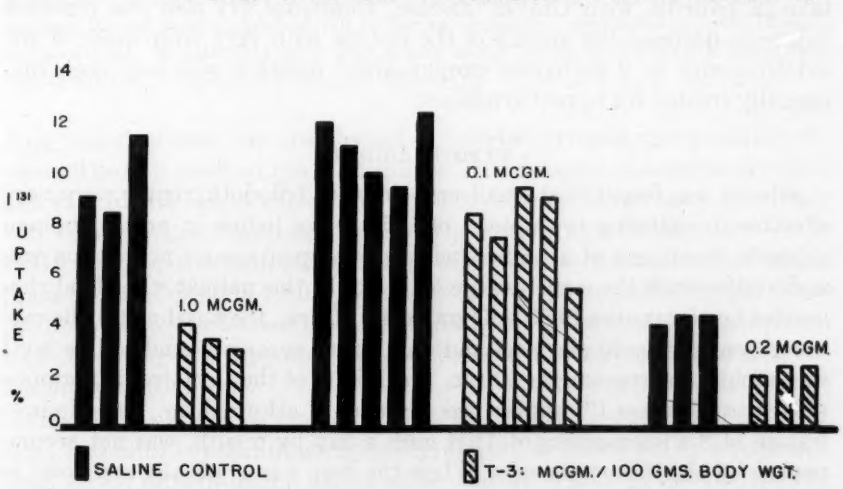

FIG. 2. Thyroidal 24-hour uptake of I^{131} (percentage of tracer dose) in saline-control guinea pigs (black columns) and in animals given triiodothyronine intraperitoneally for five days (cross-hatched columns).

peritoneal injection of a 1-microcurie dose of I^{131}, was found to reduce the 24-hour uptake of the isotope by more than 50 per cent, as shown in the first experiment. A dose of 0.1 microgram of triiodothyronine per 100 grams of body weight, given intraperitoneally, reduced the I^{131} uptake of the treated animals, as compared with the untreated controls, by about one third. In a third group of animals, which for some reason had controls with a very low I^{131} uptake, the administration of 0.2 microgram of triiodothyronine per 100 grams of body weight produced approximately a 50 per cent reduction in uptake of I^{131}. Studies on the possibility of using this method for the preparation of animals to be given TSH are under way. The effective dosage per 100 grams of body weight in the guinea pig, namely, 0.2 microgram, is of the general order of magnitude of the dosage found by Gross and Pitt-Rivers (4) to be effective in the prevention of thiouracil goiter in the rat, namely 0.32 and 0.64 microgram. These studies seem to indicate that sensitivity to TSH can be increased by such preparation. This is analogous to the findings of Querido (5), who was able to increase the sensitivity of mice to TSH by pretreatment with iodinated casein.

HYPERTHYROIDISM

In contrast to the sensitivity of the mechanism concerned in the effect of triiodothyronine on I^{131} uptake in both animal and human subjects with normally functioning thyroid glands, Werner (6) has reported that 2 milligrams, *i.e.*, over one thousand times our dosage, failed to suppress I^{131} uptake in patients with Graves' disease. Commons (7) also has reported failure to decrease the uptake of the isotope with very large doses of triiodothyronine in 2 euthyroid exophthalmic patients who had been successfully treated for hyperthyroidism.

HYPOTHYROIDISM

After it was found that small oral doses of triiodothyronine were very effective in reducing the uptake of radioactive iodine in normal human subjects, treatment of a patient with severe spontaneous myxedema was undertaken with the same dosage (Fig. 3). In this patient, the blood cholesterol level was over 1,000 milligrams per 100 cc., the basal metabolic rate was repeatedly −40 per cent and the serum protein-bound iodine level was about 1 microgram per 100 cc. The failure of the thyrotropic hormone (THS) to increases I^{131} uptake was evidence of athyroidism. The administration of 8.8 micrograms of TSH once a day by mouth, was not accompanied by significant changes. When the dose was gradually increased to 17.5, and later to 35 micrograms, there was a slight increase in the basal metabolic rate and definite improvement in the electrocardiogram. Over a

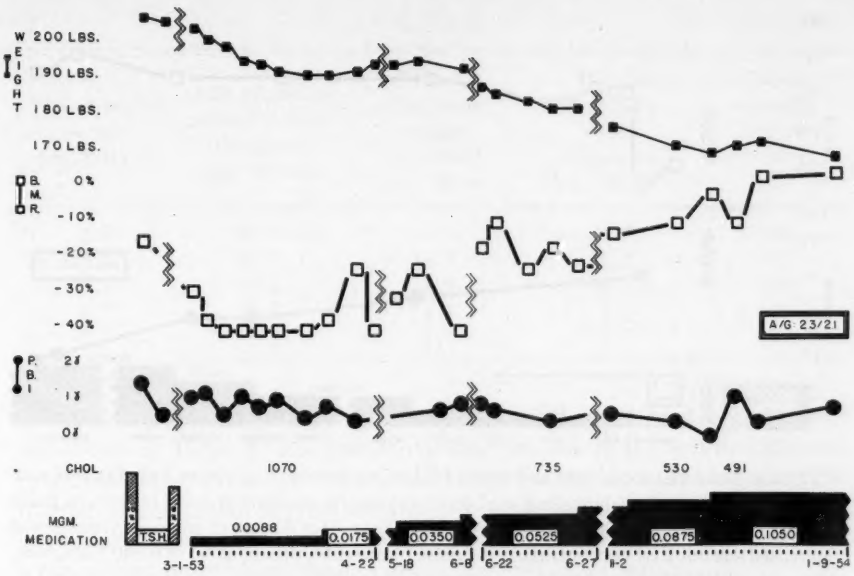

FIG. 3. Effect of triiodothyronine in an athyreotic patient (Mrs. M.K.), on weight (pounds), basal metabolic rate (per cent), and levels of serum PBI (micrograms per 100 cc.) and cholesterol (milligrams per 100 cc.). The 24-hour thyroidal I^{131} uptake is indicated by cross-hatched columns before and after three successive daily intramuscular injections of thyrotropic hormone (TSH). Solid black areas represent oral daily dosage of triiodothyronine in milligrams from March 1, 1953 to January 9, 1954. Note gradual loss of weight, gradual fall in serum cholesterol concentration, rise of basal metabolic rate to euthyroid level after a long latent period, and complete lack of any significant rise in the concentration of serum PBI.

long period of time the oral dosage of triiodothyronine was gradually increased until it reached 105 micrograms. This dosage was associated with a decrease in the serum cholesterol level, and a rise of the basal metabolic rate to normal, as shown in Figure 3. There was no elevation of the serum PBI level. Repeated samples of serum were found to contain only 1 microgram or less of protein-bound iodine per 100 cc., as measured by the Ware modification of the Barker method (8). It would seem that the medication was correcting the hypothyroid condition of the tissues without accumulating in the blood.

One of the physicians associated with this clinical research program, Dr. G.S., had mild hypopituitary hypothyroidism, as indicated by a pretreatment serum protein-bound iodine value of about 3 micrograms per 100 cc., a basal metabolic rate of −10 per cent and a radioactive iodine uptake of 28 per cent. He had taken small amounts of desiccated thyroid for many

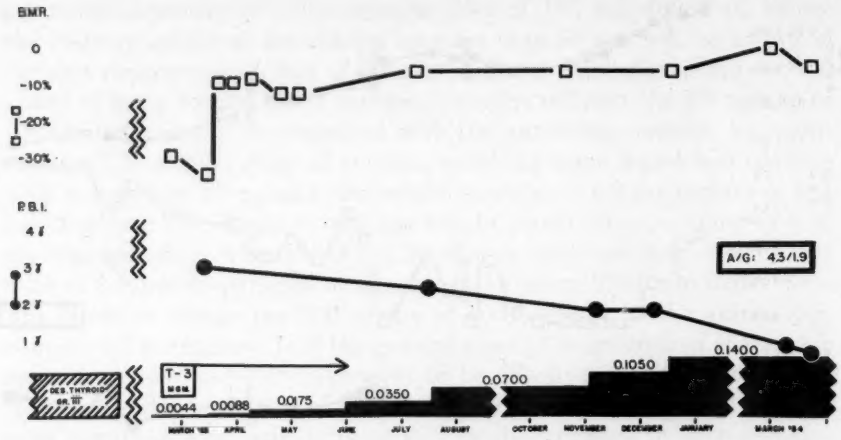

FIG. 4. Basal metabolic rate and serum PBI values in a hypopituitary, hypothyroid subject, Dr. G. S., during increasing oral daily dosage of triiodothyronine (shown in milligrams) from March 1953 to March 1954. Note that after dosage of triiodothyronine was increased, serum PBI concentration decreased until, at a dosage level of 0.140 milligram, the serum PBI value was less than 1 microgram per 100 cc.

years. After an interval of approximately two months without treatment, oral medication with small doses of triiodothyronine was begun, and the dose was gradually increased to 140 micrograms daily. There was a slight rise in basal metabolic rate, a decided improvement in subjective sensations and a gradual reduction of the serum PBI level to less than 1 microgram per 100 cc. In this patient, therefore, treatment with triiodothyronine was associated with disappearance of endogenous thyroxine from the circulating blood.

The lack of measurable serum protein-bound iodine, except in minimal amounts, might be explained by failure of detection of triiodothyronine by the Barker method. Triiodothyronine was therefore added *in vitro* to pooled serum of known protein-bound iodine content, in varying amounts, as shown in Table 1. In all samples triiodothyronine was recovered. No procedure was used to promote adsorption of the chemical by the serum protein; the determinations were made immediately after adding the triiodothyronine solution to the known serum. This test of recovery was run in triplicate on each dilution; the result represents the average of the three determinations. It would seem, therefore, that if in the examples given (Figs. 3 and 4), triiodothyronine had been present in the circulating blood, it would have been demonstrated.

As a further test, Dr. G.S. (Fig. 4) received the daily oral amount of triiodothyronine, namely 0.28 milligram, in one dose. Blood samples were

TABLE 1

PBI of pooled serum (μg./100 cc.)	PBI of pooled serum+triiodothyronine (μg./100 cc.)	Triiodothyronine iodine recovered (μg./100 cc.)	Triiodothyronine iodine calculated (μg./100 cc.)	Triiodothyronine iodine recovered (%)
5.7	17.3	11.6	14.0	83
5.7	16.7	11.0	11.2	97
5.7	11.3	5.6	5.6	100
5.7	8.8	3.1	2.8	111
5.7	8.4	2.7	2.2	123

drawn at intervals thereafter. The values before and after this treatment are shown in Table 2. The possibility that the rise in the serum PBI level (about 0.5 microgram per 100 cc.) might have been due to the ingested 280 micrograms of triiodothyronine cannot be excluded. Immediate absorption of this large dose and its retention in the blood stream would have produced a concentration of about 6 micrograms per 100 cc. of serum.

ELECTROCARDIOGRAPHIC CHANGES

In association with the extraordinary maintenance of tissue euthyroidism without elevation of the serum protein-bound iodine level, there is evidence of a significant change in the electrocardiogram, as demonstrated

TABLE 2. SERUM PBI VALUES OF DR. G. S. BEFORE AND AFTER INGESTION OF 0.280 MILLIGRAM OF LEVO-TRIIODOTHYRONINE

Time	Serum PBI (μg./100 cc.)	Inorg. iodine (μg./100 cc.)	BMR %	Pulse	Serum A/G (Gm.%)
Before treatment	0.7	2	−6	70	4.0/1.9
After treatment:					
10 minutes	1.0	1			
20 minutes	0.9	1			
30 minutes	0.9	1			
1 hour	1.2	1	+6	72	
2 hours	1.7	2	+4	68	
4 hours	1.5	2	−6	70	
7½ hours	1.5	4			
12 hours	1.4	4			4.5/1.9
26½ hours	1.2	2			4.4/1.8

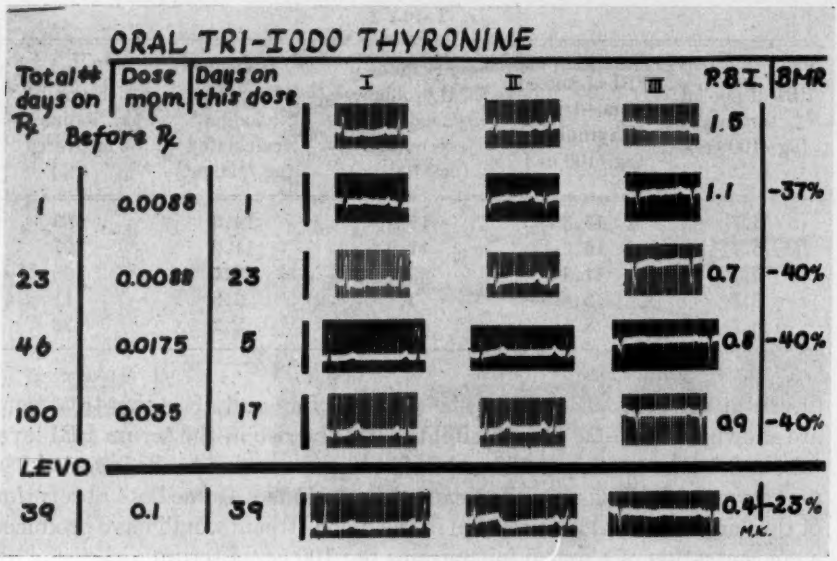

FIG 5. Electrocardiographic findings in three standard leads in the athyreotic patient (Mrs. M.K., represented in Figure 3) according to amount and duration of triiodothyronine dosage. On the forty-fifth day of total medication and the fifth day of dosage with 0.0175 milligram, electrocardiographic changes were present at a time when the serum PBI level was 0.8 microgram per 100 cc. and the basal metabolic rate −40 per cent. On the thirty-ninth day of subsequent medication with 0.1 milligram daily of sodium levo-thyroxine by mouth the electrocardiogram indicated a decrease in potential, the serum PBI level was 0.4 microgram per 100 cc., and the basal metabolic rate was −23 per cent.

in Figure 5. After forty-six days of therapy with diiodothyronine and after a dose of 17.5 micrograms per day had been given for five days, the basal metabolic rate was −40 per cent, the serum PBI level was 0.8 microgram per 100 cc., and the electrocardiogram indicated a definite increase in electrical potential and showed upward deviation of the T-waves. These changes were still more definite, approaching normal, after one hundred days of therapy, when the basal metabolic rate was still −40 per cent. Figure 6 shows similarly dramatic effects; after seventy days of therapy with a dose of 35 micrograms per day, the serum PBI level was 1.8 micrograms per 100 cc. and the electrocardiogram was normal.

SUMMARY

Maximum depression of the radioactive iodine uptake of normal subjects was produced by a single oral dose of triiodothyronine, 8.8 micro-

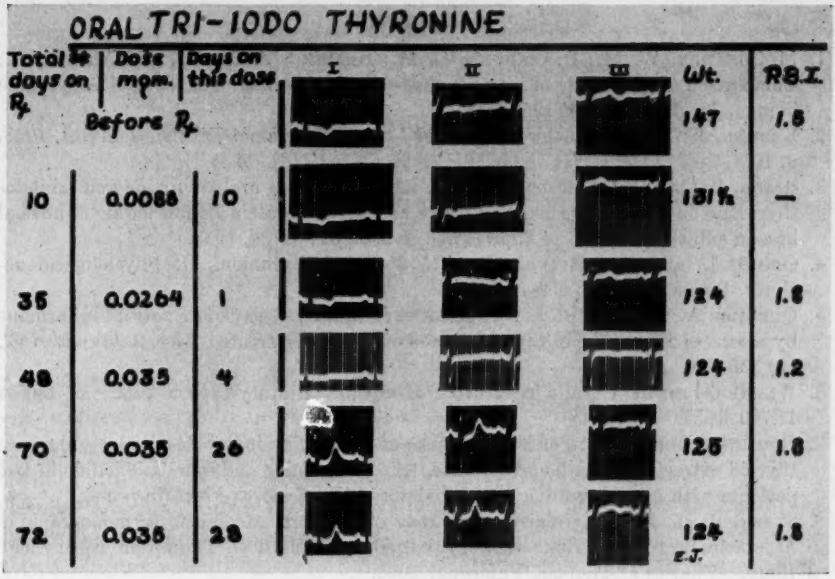

FIG. 6. Electrocardiographic findings in three standard leads during triiodothyronine administration in the case of Mrs. E.J., an athyreotic patient who had cardiovascular disease with congestive failure and Cheyne-Stokes respiration.

grams per day. This physiologic TSH hypophysectomy was also demonstrated in guinea pigs and may serve to increase the sensitivity of the animal when it is used in the bioassay of TSH. Euthyroidism was produced in myxedematous patients by small oral doses of triiodothyronine, without elevation of the serum protein-bound iodine level. Disappearance of thyroxine from the blood during triiodothyronine therapy, as indicated by depression of the serum protein-bound iodine level, was produced in a patient with hypopituitary hypothyroidism. Failure to demonstrate an increase in the concentration of serum protein-bound iodine in these studies was apparently not due to inability to obtain precipitation of triiodothyronine (if present) by the Barker method, as satisfactory recovery tests in *in vitro* studies indicate. The administration of a large single dose to the patient with hypopituitary hypothyroidism was not followed by a significant elevation of circulating protein-bound iodine and there were no definite changes in basal metabolic rate or pulse rate. The full pharmacologic action of the small dosage given, as shown by the normal electrocardiogram, preceded euthyroidism.

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FACTORS GOVERNING THE DEVELOPMENT OF THE CHICK EMBRYO THYROID

II. CHRONOLOGY OF THE SYNTHESIS OF IODINATED COMPOUNDS STUDIED BY CHROMATOGRAPHIC ANALYSIS*†

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THERE is an old saying that "what goes up must come down." This expression could be paraphrased to read "what goes up might come down," and applied to a graphic representation of the fascinating process of biologic differentiation as exemplified in embryonic development. Do some tissues "slip back down the embryonic stairway"? If so, does this fact play a significant role in neoplasia, either benign or malignant? Is there any way to induce such tissues to redifferentiate for a second, or many times? Is it possible even to hasten the normal embryonic process of differentiation? What role, if any, is played by so-called tissue organizers? Is the influence of such organizers limited to the embryonic era or could undifferentiated neoplastic cells perhaps be redifferentiated under such influence? Could, in fact, normal adult cells even be over-differentiated by such exposure?

This communication does not answer any one of the foregoing questions. The results reported do, however, suggest that if modern techniques of investigation are brought fully and energetically to bear upon the problem of differentiation, we may reasonably anticipate considerable progress towards better understanding of these and related matters.

We have outlined in an earlier publication (1) some reasons for believing that answers to the questions just raised might be applicable to the solution of some urgent clinical problems, particularly those related to cancer. Because of the special and selective way in which the thyroid gland utilizes iodine, we have chosen to attempt a characterization of the "embryonic stairway" up which the primitive thyroid gland must come in

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order to be able to perform its peculiar role in the body. It would seem to be a safe assertion that a study of this kind would not be possible without radioactive iodine, or for that matter, without chromatography. The text of this report will deal primarily with the facts that 1) the differentiation of the embryonic thyroid gland of the chick is a stepwise process, 2) dedifferentiation can and does occur, and 3) as suggested by Wegelin in 1926 (2) some adenomas of the human thyroid bear very strong resemblance to embryonic thyroids which have only partially reached their ultimate goal of full specialization.

METHODS AND MATERIALS

Fertile eggs of a hybrid strain resulting from a cross between New Hampshire reds and white leghorns, were obtained from the experimental farms of Texas A & M College. Although these eggs were collected there every three hours to minimize lack of uniformity in incubation time, there was another factor which often contributed to some uncertainty in this regard. When shipments were delayed in warm weather (both factors operating often in Texas) some incubation occurred before the eggs could be transferred to the incubator and start from "zero" time. Although this could generally be detected, because the embryos would be larger than normal at any given age of incubation, nevertheless there were some discrepancies due to this factor. Complete runs at every point on the curve had to be repeated several times (more than 600 separate radiochromatograms were made to complete this study in order to obtain reproducible values). When working with very young embryos it was necessary to administer as much as 100 microcuries of I^{131} per egg and to pool at least a dozen thyroids in order to obtain sufficient tissue. In the case of older embryos, as little as 5 microcuries of I^{131} was required to obtain comparable results. Even at the time of hatching, at least 6 thyroids were required for representation as one point on the curve.

Points on the curve were first obtained for each twenty-four hours from $7\frac{1}{2}$ days through 1 day after hatching. Then attention was concentrated upon smaller and smaller intervals of time, until finally points were obtained for every three hours of incubation during the period of greatest interest and most rapid change (from $7\frac{1}{2}$ through $10\frac{3}{4}$ days).

The complete procedure was as follows: I^{131}, in the doses previously mentioned, was injected into the air sac of the egg through a small triangular window, which was then resealed. The egg was returned to the incubator for twenty-four hours, following which the thyroid glands were removed and counted in a well counter. They were then denatured in a solution of 80 per cent alcohol to which 1 per cent trichloracetic acid had been added. After twelve hours in this solution, the tissue was washed twice with additional 80 per cent alcohol, and the washings were added to the denaturing solution which

was saved for counting and for chromatography. The tissue was then hydrolyzed over a boiling-water bath for sixteen hours in 0.2 ml. of 2N sodium hydroxide, cooled, acidified with 3N sulfuric acid to pH3, and extracted with petroleum ether. It was extracted again with four washings of butyl alcohol. The ether extraction was performed to remove some iodinated lipids and other ether-soluble substances, which not only interfered with the running of the chromatograms but also tended to localized in the same region as that occupied by thyroxine.

The trichloracetic acid "extract," the ether extract, and the butanol extract were each concentrated individually under a flow of warm air and were applied along lines near the bottoms of strips of Whatman #1 filter paper. Ascending chromatograms were made by the method of Taurog *et al.* (3) and were allowed to run for sixteen hours. After drying, the strips were placed against x-ray film and exposed for eight days. Following exposure, the film was developed and the darkened spots were used as guides in locating and bracketing the separated compounds on the filter-paper strip. These compounds were then separated from each other by cutting along the pencilled boundary lines, and the resulting smaller strips were introduced into a well counter in order to determine the amount of radioactivity present. Densitometric measurements were not attempted because of the narrowness of the limits within which blackening is proportional to radioactivity. Since only one half-life elapsed during the exposure period of the chromatogram to the x-ray film, there was always enough activity left on the paper to yield highly significant results in the counter. For a variety of reasons we chose to present the data obtained as "counts per thyroid," rather than to attempt to estimate the true quantity of each compound. It should also be noted that, in Figure 3, neither the front running material nor that which remained at the point of application has been graphed, thus accounting for the fact that the sum of the counts for the compounds which are shown therein does not equal the value represented as "total."

In the few human studies reported here, a dose of 500 microcuries of I^{131} was administered to patients with adenomas of the thyroid gland twenty-four hours prior to operation. Immediately after the surgical specimen was delivered, small bits of the adenoma and of the normal tissue were dissected out and subjected to the procedure previously outlined. Ordinarily there are more interfering materials in relatively large pieces of human thyroid than we encountered in the chick specimens, and the chromatograms were not of as high a quality. Some changes were needed in the procedure and have been made. They will form the subject of a separate communication, however.

A number of other digestion and chromatographic techniques were tried before those described in this report were selected. These discarded techniques are mentioned, together with our reasons for rejecting them, in the hope that others may be spared wasted time. Barium hydroxide, when used for a digesting agent, does not function as effectively as sodium hydroxide. Enzymatic digestion by the use of trypsin works well most of the time but, in our hands, was not as reproducible as the sodium-hydroxide method. We ran one entire series from seven days through hatching, using trypsin digestion; the results were essentially the same as those reported in this paper. A lutidine water-solvent system not only failed to separate the compounds, but produced chromatograms in which there was considerable tailing. An ethanol-ammonia-water mixture likewise

failed to separate the compounds adequately. Using butanol-acetic acid-water we obtained chromatograms which ran sharply and separated the compounds well, but in which there was loss of iodine (as I_2) from the paper during the process of running. Also, thyroxine tended to merge into the solvent front. We had good results with the Taurog method (collidine 125 parts, water 44 parts, and ammonia atmosphere).

Reference is made later to a series of chromatographic studies (4) of embryonic chick thyroid cells which were removed from the embryo after 7 days of *in vivo* incubation and then transferred to roller-tube cultures, in which they were carried for varying periods of time. I^{131} was added twenty-four hours prior to digestion. From this point on, the technique was the same as that previously described.

RESULTS

Figures 1 and 2 give the essence of our results. It should be mentioned here, however, that although no chromatograms were made before $7\frac{1}{2}$ days, we did compare the iodine uptake of the thyroid with that of heart muscle in 5-day embryos. Even at this early stage there was as much as fifty times

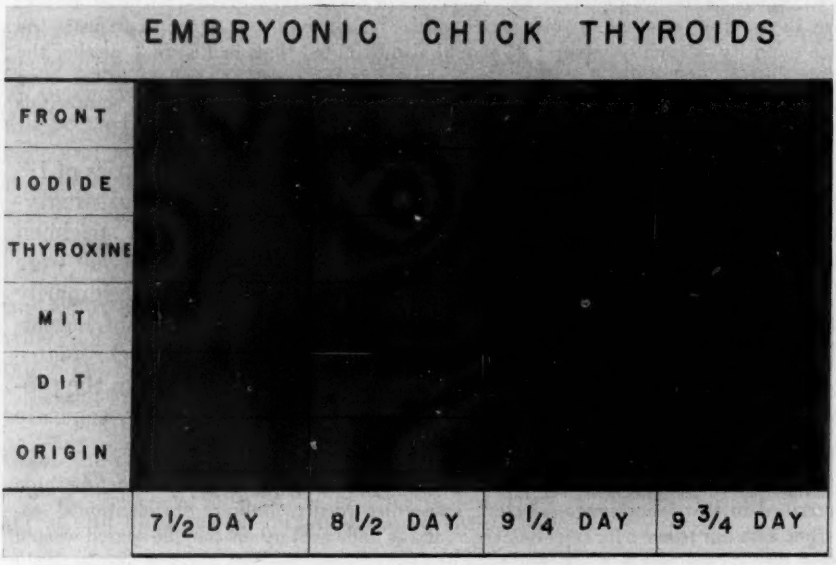

FIG. 1. Representative autoradiograms of chromatograms made at the times of incubation indicated. Note that the compounds appear in the same order in which they are formed during the artificial synthesis of thyroxine. At $7\frac{1}{2}$ days the embryonic gland is still capable only of trapping iodide. Even at $8\frac{1}{2}$ days it can form only monoiodotyrosine. Shortly thereafter, however, diiodotyrosine also appears, and at $9\frac{1}{4}$ days the first small quantity of thyroxine is seen.

more iodide in the thyroid than in the heart muscle. It is difficult to be certain of this ratio because of the problem of obtaining 5-day thyroids completely free from surrounding nonthyroidal fragments. Virtually all of the activity in these thyroids was removable by treatment with the trichloracetic-alcohol solution, indicating that little or no binding and probably no conversion to iodine had taken place. It will be seen in Figure 1 that this situation existed as late as 7½ days, and almost for another twenty-four hours. At 8½ days, however, monoiodotyrosine was first synthesized. Roughly ¾ of a day later, diiodotyrosine also appeared, and about ½ a day later the first, very faint smudge of thyroxine was seen. It was not until 10 days (Fig. 2) that thyroxine was being produced in easily detectable quantities. The reader should be cautioned not to take the time designations of these chromatograms literally, since, as was explained in the section entitled Methods and Materials, we were not always certain when incubation began. For example, there were numerous occasions on which what were considered 8-day embryos showed both monoiodotyrosine and diiodotyrosine. The chromatograms chosen for presentation here represent,

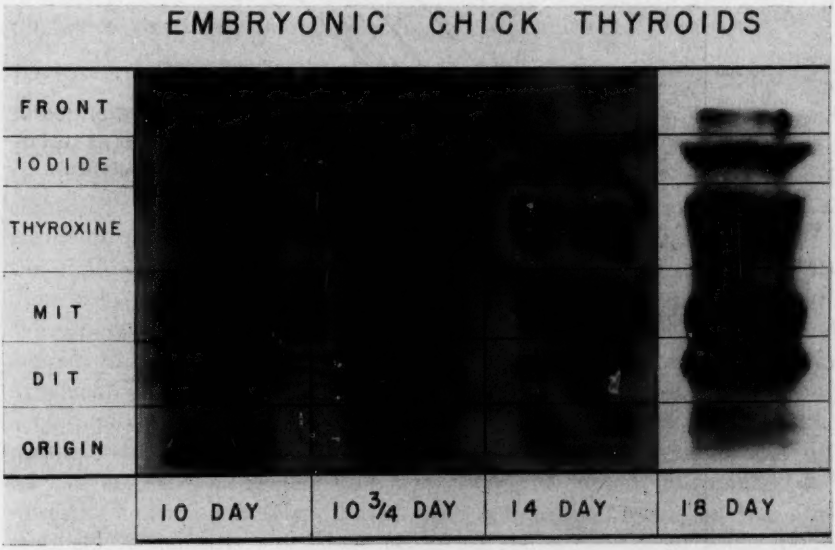

FIG. 2. Autoradiograms of chromatograms made at the times of incubation indicated. Note that the 10-day gland produces relatively large amounts of thyroxine whereas, only 6 hours earlier, this compound is barely detectable. From the tenth day on through hatching, few differences are apparent by inspection of chromatograms alone. (See Figure 3 for quantitative relationships.) If triiodothyronine is present, it is not separated from thyroxine well enough in our solvent system to be identifiable.

our most trouble-free results. After the tenth day, mere visual inspection of the chromatograms conveys the idea of no further change. Upon examination of Figure 3, however, in which our data are presented semi-logarithmically, it is apparent that the total amount of iodine increased further. Histidine is the only amino acid besides tyrosine which iodinates readily. Very small amounts of monoiodohistidine were apparent in the region of diiodotyrosine on many of our chromatograms, but this detail could not be reproduced well enough for publication. The most careful inspection failed to reveal any evidence of the presence of triiodothyronine. However, the chromatographic system employed does not separate thyrox-

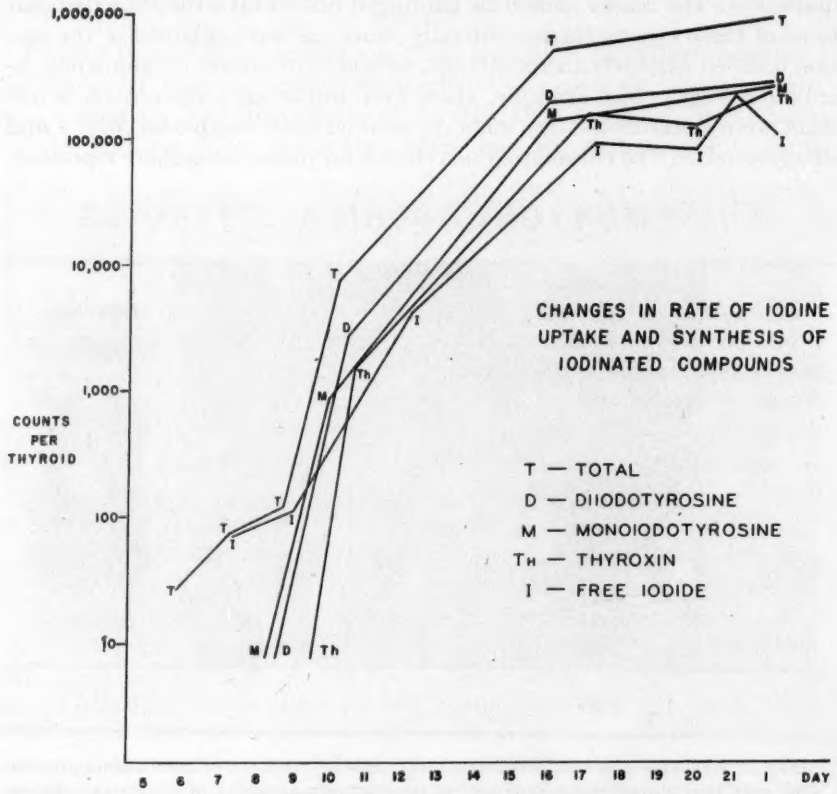

FIG. 3. This semi-logarithmic representation of the data shows the relative abundance of counts supplied by the various intermediates and by thyroxine at any given time during the period of incubation. It also draws attention to the relative rates of increase (and occasionally of decrease, as seen for thyroxine between the seventeenth and twentieth days).

ine and triiodothyronine sufficiently well to warrant the conclusion that there was no triiodothyronine in any of these tissues.

Figures 4 and 5 illustrate similarities and differences between adenomas of human thyroids and the normal thyroid tissues in which they grew. Figure 4 shows an adenoma which was very little different from its normal counterpart, the chief alteration being that the quantity of thyroxine present in the adenoma was diminished. The appearance of this chromatogram is strongly reminiscent of that of the chick embryo at roughly 9¾ days, at which point thyroxine has barely appeared. Figure 4B shows that the adenoma was primarily a micro-follicular one, but with some earlier elements also present. Figure 5, on the other hand, shows an adenoma which was somewhat less efficient than that in Figure 4, being able to produce only monoiodotyrosine. However, the normal gland from which it was removed was functioning as it should. Figure 5B shows that this adenoma was a micro and macro-follicular tumor. Theoretically it should have been not less, but more biochemically efficient than the adenoma shown in Figure 4. We have no explanation for the fact that it was not. A similar study of the functional capacities of 50 adenomas and thyroid cancers will comprise the text of a separate communication.

DISCUSSION

Blanquet *et al.* (5), using a different method of digestion (trypsin) and a different chromatographic system (butanol and acetic acid) presented the results of similar determinations made on the twelfth, fifteenth and eighteenth days of incubation. They found a very small amount of thyroxine on the twelfth day and were unable to demonstrate free iodide on the eighteenth day. This latter finding is probably related to loss of iodide from the paper during chromatography in an acid solvent system. Waterman and Gorbman (6), studying the thyroids of fetal rabbits, found a point at which monoiodotyrosine and diiodotyrosine were present but at which thyroxine had not yet been formed. Gorbman *et al.* (7), reported a chromatographic analysis of bovine fetal thyroids, and compared the results with a similar analysis of the maternal glands and of the thyroid of an ox. The fetal glands were obtained late in pregnancy, and were not only completely differentiated (producing thyroxine) but were more active than the thyroids of either the mother or the ox. Since iodine accumulation by the fetal bovine thyroid begins on or before the sixtieth day, this study sheds little light on the early stepwise portion of thyroidal differentiation.

We are aware of the possibility that some of the intermediates which we found at early stages of incubation may have been artifacts, in the sense that, if enough iodine is present, some nonspecific iodination of tyrosine

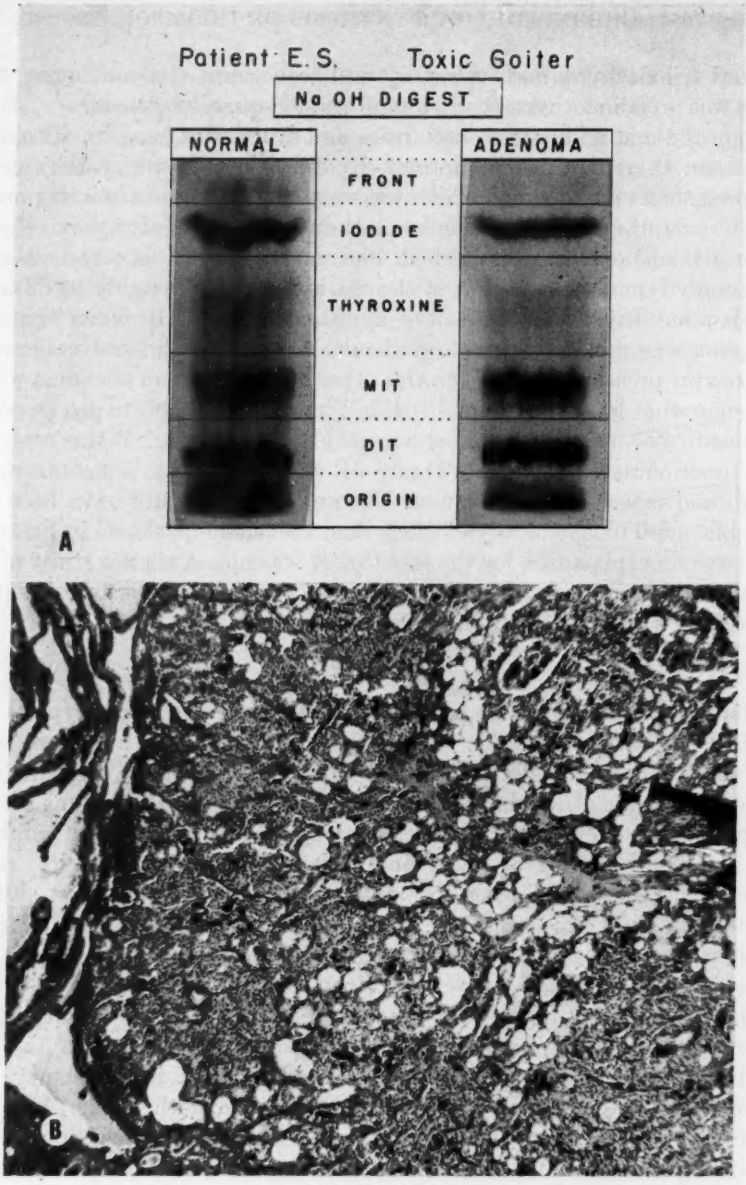

FIGURE 4

A. Radiochromatograms of "normal" and adenomatous thyroid tissue removed from a 45-year-old male twenty-four hours after administration of 500 microcuries of I^{131}. The adenoma is capable of nearly full function but does not contain as much thyroxine as the control. It is best compared with the chick embryo at $9\frac{3}{4}$ days of incubation.

B. Photomicrograph of thyroid tumor represented in **A.** This is an early microfollicular adenoma.

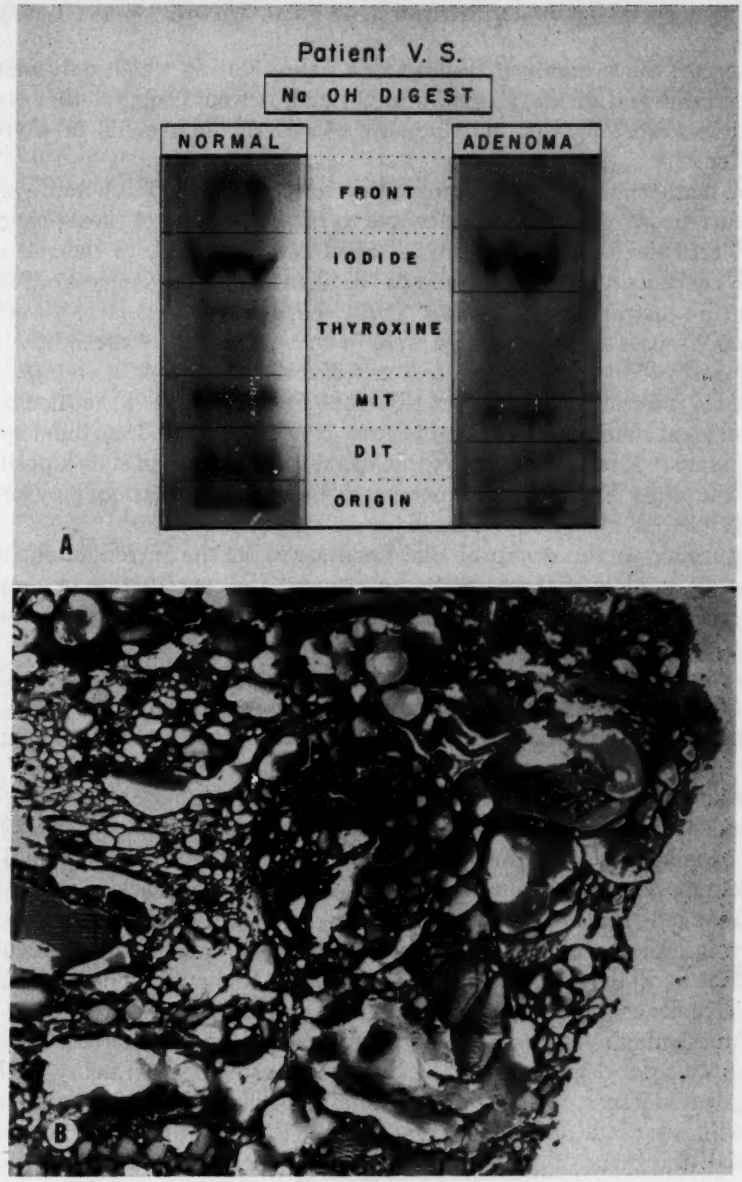

FIGURE 5

A. Radiochromatograms of "normal" and adenomatous thyroid tissue removed from a 28-year-old female twenty-four hours after administration of 500 microcuries of I^{131}. This adenoma can trap iodide and can make monoiodotyrosine, but can go no further with the synthesis of thyroxine. This degree of function compares almost exactly with that of the chick embryo thyroid after 8½ days of incubation.

B. Photomicrograph of thyroid tumor represented in **A.** This is a micro- and macrofollicular adenoma.

may occur. Since, however, there was a period during which only monoiodotyrosine and diiodotyrosine were formed, it would suggest that these substances were present only because of orderly increments in thyroid function.

The many changes in the slopes of the curves of Figure 3 are impressive. Our current investigations are devoted to the elucidation of these phenomena. Particular attention might be called here, however, to the rise and fall in concentration of iodide and to the slight fall in the concentration of thyroxine just prior to hatching. These changes were regularly observed on many separate occasions and were in that phase of the curve in which statistical validity is at its greatest in terms of the number of counts.

Also of interest is the fact that thyroxine first appeared at the time most workers feel colloid manufacture begins. It is conceivable that diiodotyrosine units are arranged in the peptide spiral of the colloid in such a position that the ether linkage which results in thyroxine formation may occur easily.

Returning to the group of questions asked in the introduction, it is tempting, in view of these results, to attempt the construction of a unifying concept which would relate three phenomena, at least in a general way. The first is embryonic differentiation of the thyroid. The second is the occurrence of adenomas and cancers which have limited synthesizing abilities (and which resemble morphologically some embryonic stages). The third involves the findings of Dr. Gonzales with respect to what is apparently de-differentiation *in vitro*. As a working hypothesis, we should like to believe that each step represented by the developing chick embryo is dependent upon single or mutliple enzyme systems, each of which is probably specific in nature. Each of these enzyme systems in turn would depend upon a gene, or genes, for its appearance. Going back one step further, one may speculate that an "organizer" of some kind is responsible for selecting and "turning on" the appropriate gene and may even regulate the time at which the gene begins to function. It should be borne in mind that all cells contain all genes (for a given species) and that relatively few are functioning in any one specialized tissue such as the thyroid. We look hopefully toward workers in other fields to cast light upon many of these complicated issues, but in the meantime, feel that additional studies along these lines are indicated. With respect to the thyroid at least, we now know some of the "when" of embryonic developmental features. The "what," "why," "where," and "how" remain to be explored.

SUMMARY AND CONCLUSIONS

1. The biochemical differentiation of the chick embryo thyroid gland is a gradual process, similar in duration to the morphologic differentiation,

although not necessarily initiated or fostered by the same factors.

2. This differentiation appears to take place in steps, between some of which there are long intervals. Between others, the spacing is a matter of only a few hours.

3. The primitive gland is able to trap iodide at an early age. The exact time has not been determined, but it is at least by the fifth day.

4. After about 8½ days of incubation, monoidotyrosine, but not diiodotyrosine can be formed. This same situation was observed in cells growing in tissue culture as well as in some human adenomas.

5. Less than one day later, the ability to form diiodotyrosine is acquired.

6. Whereas monoiodotyrosine or diiodotyrosine, or both, can be formed prior to the advent of colloid production, thyroxine does not appear until the first colloid droplets are seen.

7. The limitations observed in the capacities of some human thyroid adenomas to process iodide through all of the steps required to produce thyroxine are similar to the various limitations seen during the process of embryonic differentiation in the chick thyroid.

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IODINATED COMPOUNDS IN THE SERUM, DISAPPEARANCE OF RADIOACTIVE IODINE FROM THE THYROID, AND CLINICAL RESPONSE IN PATIENTS TREATED WITH RADIOACTIVE IODINE*†

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THERE is general agreement that most of the organically bound radioiodine in the blood after therapeutic doses of I^{131} is comprised of thyroxine. The presence of significant amounts of butanol-insoluble organic iodine, diiodotyrosine, monoiodotyrosine (1), and triiodothyronine (2, 3) has been reported. It is not definitely known whether the appearance of any one, or all of these compounds results from the effects of radiation on the thyroid gland. It is the purpose of this report of work in progress to describe studies dealing with possible relationships between the iodinated compounds appearing in the serum, the rate of disappearance of the radioiodine from the thyroid, the amounts of radiation delivered to the gland, and the clinical response to therapy.

METHODS

Chromatographic fractionations of serum radioiodine extractable in *n*-butanol were made after 34 therapeutic doses of I^{131} in 31 patients. Most of the patients had Graves' disease, but 1 with toxic nodular goiter, 1 with metastatic thyroid cancer following surgical thyroidectomy, and 2 with thyroid remnants following surgery for thyroid cancer are included.

Before treatment, patients were studied clinically and also with determination of the basal metabolic rate, measurement of the thyroidal uptake of radioiodine, and estimation of the size and character of the thyroid gland. Following the administration of the therapeutic dose of I^{131} the urinary excretion of I^{131} was observed for seventy-two hours

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‡ Research Fellow of the American Cancer Society.

and the amount of radioiodine in the thyroid gland was determined at frequent intervals for at least one week, and in most instances for thirty days. Signs of increased toxicity, tenderness, and changes in the size or consistency of the thyroid were noted. The therapeutic result was judged by repeated metabolic rate determinations, radioiodine tracers, and clinical evaluation.

Samples of blood were collected at frequent intervals, in some instances daily, and were allowed to clot in a tube containing a crystal of sodium thiosulfate.[1] Within a few hours the serum was extracted twice with double volumes of *n*-butanol and twice with single volumes. The efficiency of the extraction was measured by counting radiation in the serum before, and in the residue after, the final separation. Control extractions performed after adding I^{131}-labeled compounds to serum gave a recovery of 82 per cent of thyroxine, 80 per cent of triiodothyronine, 76 per cent of iodide, 85 per cent of monoiodotyrosine, and 49 per cent of diiodotyrosine. Good recovery of iodide was possible, because no alkaline wash was used (6). The butanol fractions were combined and immediately evaporated to dryness on a rotary vacuum still. The residues were resuspended in a small volume of *n*-butanol just before application to the chromatographic paper.

Chromatograms were made on paper strips with a solvent consisting of the supernatant fluid from a 4 : 1 : 5 mixture of *n*-butanol, dioxane, and 0.6 N ammonium hydroxide in water. The method was modified from the second part of the two-dimensional system described by Gross and coworkers (7). These chromatograms were run in a descending fashion, to extend the distance of flow and thus give a wide separation of the compounds.[2]

One or several carriers of thyroxine, triiodothyronine,[3] diiodothyronine, diiodotyrosine, and monoiodotyrosine were frequently added to the paper strips at the point of application. Carriers were used to lend assurance to the identification of the labeled compounds from the serum. The positions of these carriers were identified by the application of ninhydrin®. In a similar fashion sodium iodide was used and identified by its color reaction with palladium chloride. The descending chromatogram was permitted to develop to a length of about 18 inches. It was cut into ¼-inch bands for counting under a mica window counter.

Counting was carried out for a minimum interval of one minute and for a total of at least 128 counts. Levels of radiation greater than twice that of the background (20 counts per minute) were regarded as significant, provided three or more adjacent bands met this criterion. A semiquantitative estimate of the compounds present in the chromatogram was made by calculating the areas under the curve after subtracting 10 counts per minute from the net counts (to eliminate areas due to the variation in the counting of background). In the last 15 patients studied, the total serum radioiodine concentration was compared to that of an aliquot of the dose, so that the chromatographic yield of compounds might be related to the original amount administered. Using this chromatographic system, the reproducibility of the method with or without carriers was fairly good, provided the carriers were limited to small amounts.

Estimates were made of the speed of conversion of iodide to organically bound iodine in the serum. In 20 of the patients investigated during the first part of this study, fractionation of the serum I^{131} was carried out by Somogyi's reagent. The radioactivity in the precipitate was interpreted as representing that portion of the iodide converted to

[1] This was added to prevent oxidation and to avoid interchange of radioiodine with nonradioactive compounds (4, 5).

[2] Full details of the method will be given in a subsequent publication.

[3] Furnished through the courtesy of Smith, Kline and French Laboratories.

the organic form (8). Following the 14 most recent treatments with I^{131}, the conversion ratio was based on the sum of the I^{131}-labeled organic compounds recovered by the chromatographic fractionation after butanol extraction of the serum.

RESULTS

During the investigation 39 therapeutic doses of radioiodine were administered, 4 in connection with the treatment of cancer and 35 for hyperthyroidism.[4] Of the doses administered for hyperthyroidism, 3 produced myxedema, 13 produced a euthyroid state before three months had elapsed, and 7 produced a euthyroid state after three months. After 4 of the treatments there was only fair improvement, and after 3 there was a poor response, necessitating retreatment. The time interval following 5 of the doses has been insufficient to permit classification of the clinical response.

One or more determinations of the thyroidal uptake of a tracer dose were made subsequent to 23 treatment doses (Table 1). In patients who became myxedematous or became euthyroid slowly, results of the subsequent tracer studies were within or below the normal range. Among the patients who had either a fair or a poor clinical response, only 1 had a normal uptake. Following 8 of the treatments there was a prompt return to the euthyroid state in patients who had subsequent tracer studies. In spite of the euthyroid state, 3 patients still had an elevated uptake at six months (J.C.), five months (R.M.), and four months (H.B.) after treatment. In Patient R.M. the uptake finally became normal at eight months, although the clinical response had been judged good at one month. This paradox has been reported previously in an occasional case (9), but is the converse of the observations in a few patients reported by Chapman and coworkers (10).

TABLE 1

Clinical result of I^{131} treatment	Subsequent thyroidal uptake of I^{131}	
	Normal or low	Elevated
Myxedema	3	0
Euthyroid before 3 months	5	3
Euthyroid after 3 months	7	0
Fair or poor	1	4

The size and weight of the thyroid gland were estimated by careful palpation by one or both of the authors. The thyroid was judged to have become normal or not palpable in 17 instances, much smaller but not normal

[4] No chromatographic studies were made after administration of 5 of the treatment doses.

in 8 instances, only slightly smaller in 2 instances, and unchanged or actually larger than previously in 6 instances. In 2 cases there was no long-term follow-up, and 4 others were excluded because the I^{131} was administered for the treatment of cancer. Although it is well known that it is difficult to estimate the size or weight of the thyroid, a fair correlation was found between clinical response and changes in gland size.

Early in this series of observations it was suspected that a rapid decline of the radioactivity in the thyroid gland should herald a good therapeutic

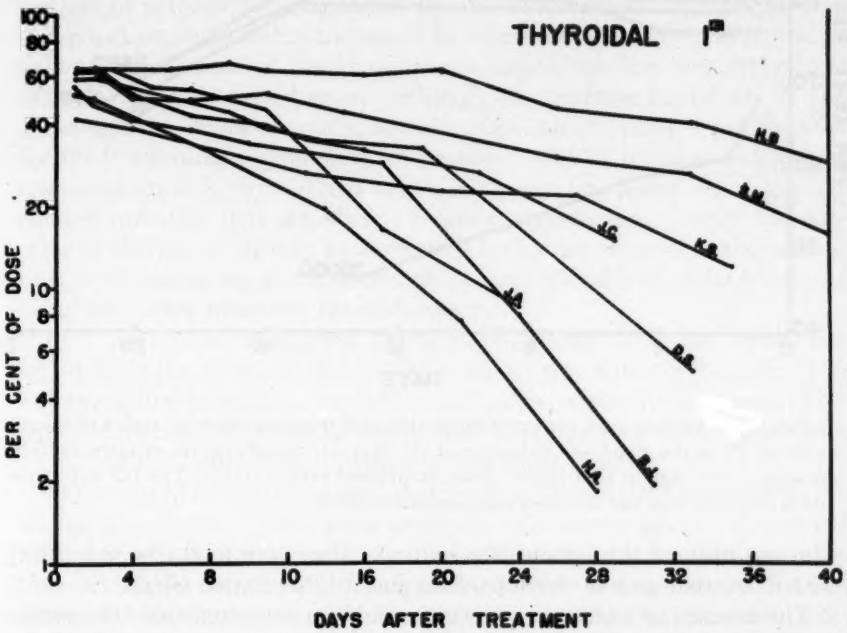

FIG. 1. Changes in the amounts of thyroidal I^{131} in selected patients. Therapy was promptly effective in all except B.J., who responded poorly. H.A. and S.M. became myxedematous. The break in the curve toward a more rapid rate of loss is thought to herald a good therapeutic result.

response. Subsequent investigation of those patients with a rapid decline in radioactivity revealed both good and poor responses. Upon further inspection of the curves, it was found that there was an increased rate of loss of I^{131} after about two weeks in those patients with a good clinical result (Fig. 1). One patient (B.J.) who had such an increase, however, had little clinical effect from the treatment. Thus it was not necessarily the downward course of the curve which gave evidence of radiation effect, but a break toward a steeper decline which indicated damage to the gland. A steep de-

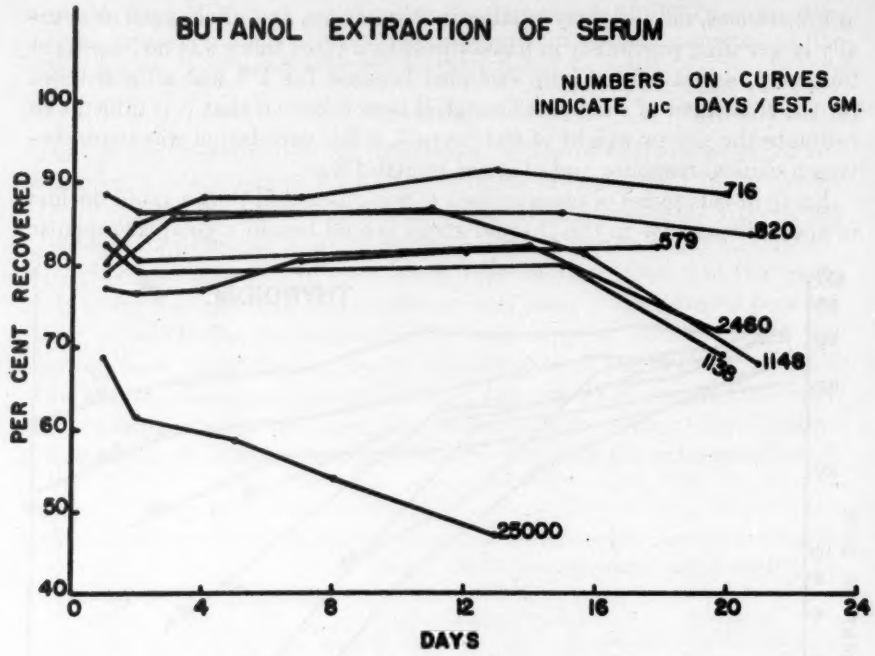

FIG. 2. Microcurie-days per gram were estimated from planimetric studies of microcuries of I^{131} in the gland and palpation of the thyroid. Sample curves of extractability are shown and suggest that higher doses diminished extractability. The fall with time also is evidence that this decrease was a radiation effect.

cline maintained throughout the course of the curve might be associated with a severe degree of thyrotoxicosis and little radiation effect.

The amount of radioactivity which could be extracted from the serum with *n*-butanol at pH 6–7 was diminished in patients who received the most radiation to the thyroid, whether for Graves' disease or for cancer. These results are similar to those reported by Robbins and co-workers (1) after large doses administered for cancer. The extractability decreased as time passed—in 1 instance to below 50 per cent of the total, as shown by sample cases in Figure 2. Since in the control experiments the recovery of labeled compounds added to serum was greater than 70 per cent (with the exception of diiodotyrosine), this decrease must have been due to the accumulation of butanol-insoluble compounds such as thyroglobulin, as reported by Robbins (11). The fact that extractability was related to the size of the dose and the time after administration of the dose suggests that the change in extractability was the effect of radiation on the thyroid.

The total radiation delivered to the thyroid was estimated from plani-

metric studies of the curve representing the microcuries of I^{131} found in the gland at frequent intervals following the administration of the treatment dose. The microcurie-days as derived by this method were divided by an estimate of the weight of the thyroid; the resultant values ranged from 500 to 24,000 microcurie-days per estimated gram. This calculation probably is a better index of thyroidal radiation than are indices based on a single measurement of 24 or 48-hour uptake, because the method described here allows for variations in the rate of loss of I^{131} from the gland. Using this method of analysis, the calculated amount of radiation can be related to the reduction in the size of the gland. In all instances in which there was no reduction in the size of the gland, the radiation was less than 900 microcurie-days per estimated gram. Although the decrease in the size of the gland and the clinical response seemed to go hand in hand, 1 patient who received radiation (calculated) amounting to 1,970 microcurie-days per estimated gram of thyroid had only a fair clinical response. From the calculated radiation it is not always possible, therefore, to predict the outcome of therapy. This may be accounted for by inaccuracies in the estimation of the size of the gland, especially in patients with irregular masses of recurrent tissue following thyroidectomy.

In 7 of 15 patients studied, the level of radioiodide in the serum was found to be less after one or two days than it was after three to six days. Following this secondary rise the level of iodide gradually decreased. With only 1 exception, the patients who showed this phenomenon were those who converted the radioiodide to organic forms most rapidly.

Following 15 of the treatments there were sufficient data to calculate the changing amounts of circulating thyroxine as a percentage of the dose per liter of serum. Three types of thyroxine curves were seen. In 4 cases the thyroxine level rose rapidly to about 1 per cent of the dose per liter of serum. Such a curve is shown in Figure 3. This occurred in the patients with a high rate of conversion of iodide to organic forms. In 4 cases there was a less rapid rise to a moderate amount of thyroxine. In 7 cases the level of serum thyroxine was less than 0.5 per cent of the dose per liter. Patients with a low thyroxine level had a low rate of conversion, with the exception of 1 who was treated for toxic nodular goiter. This patient had a conversion ratio of 88 per cent at twenty-four hours, but the largest amount of thyroxine in the serum was only 0.23 per cent of the dose per liter at fifteen days.

Although the described system of chromatography does separate thyroxine from triiodothyronine, it does not separate triiodothyronine from diiodothyronine. Since triiodothyronine is known to appear in the circulation (3) and since diiodothyronine has not been identified in serum, it is presumed that a labeled compound with an R_f value greater than that of

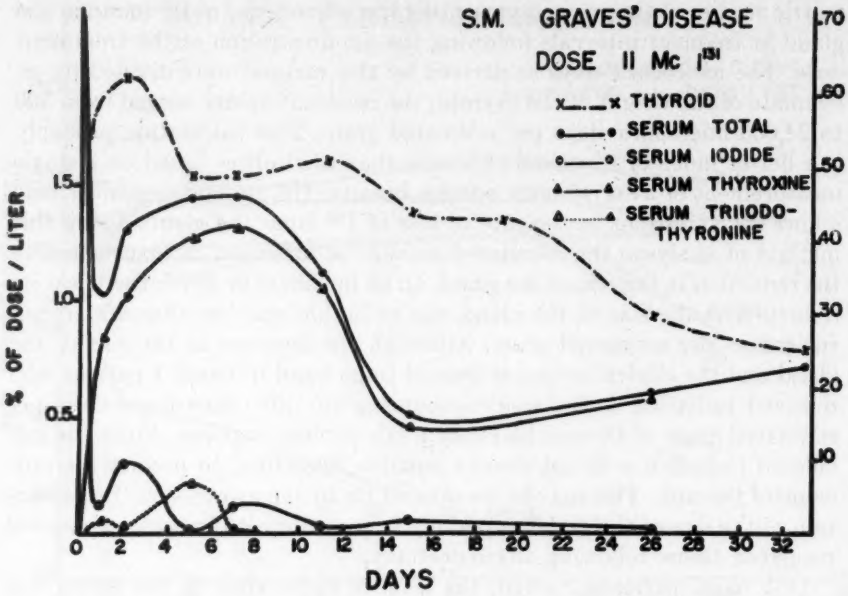

FIG. 3. A rapid increase in the level of serum thyroxine was demonstrated in this patient. Variations in the thyroxine level may parallel changes in the thyroidal I^{131}, the former decreasing when the latter remains unchanged. An initial depression of circulating iodide is also demonstrated.

thyroxine but not approaching the solvent front is triiodothyronine (Fig. 4). Triiodothyronine was detected in the serum of 19 patients after 21 of the doses of radioiodine. Amounts over 3 per cent of the radioactivity in the serum were thought to represent more than a trace, and were present in 15 patients (Table 2). The largest amount detected was 19 per cent in a

TABLE 2

Diagnosis	No. of patients	No. of patients showing unusual compounds in serum		
		Tri	Mono	Di
Graves' disease	27	17	11	3
Cancer; no thyroid remaining	1	1	1	1
Cancer; some normal thyroid remaining	2	1	2	2
Toxic nodular goiter	1	0	0	0
Totals	*31*	*19*	*14*	*6*

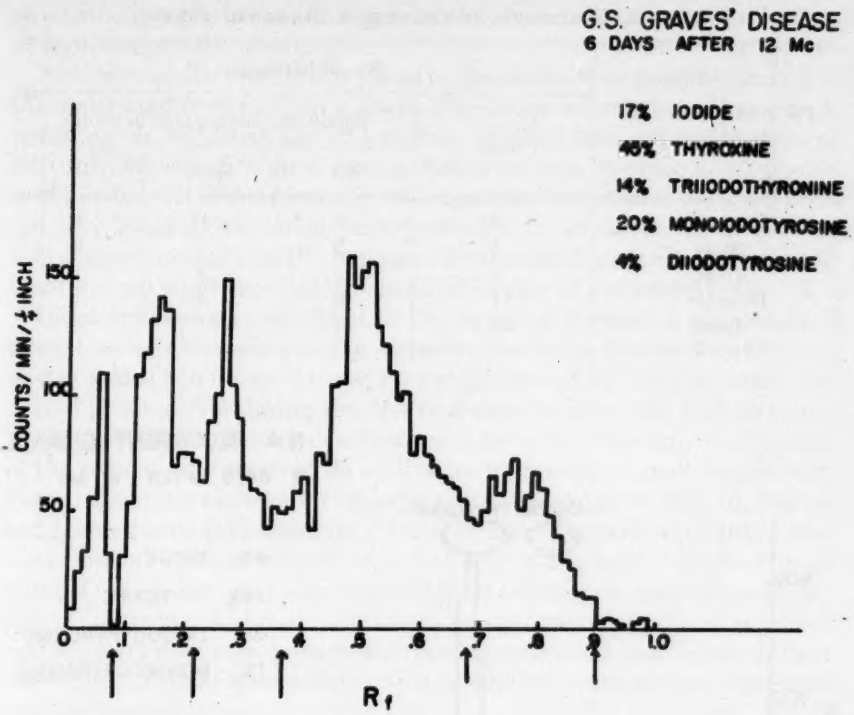

FIG. 4. The chromatographic fractionation of the butanol extract of the serum was made roughly quantitative by calculating areas under the peaks. The arrows indicate the selection of dividing lines between compounds.

patient receiving 35 millicuries of I^{131} to destroy a very small remnant of normal thyroid left behind during an operation for cancer.

There are three reasons for believing that the presence of triiodothyronine in the serum is not necessarily an effect of radiation. First, among the 19 patients in whom triiodothyronine was detected, it was found in 13 as early as twenty-four hours following the administration of the I^{131} (Fig. 5), and in all but 1 patient before the end of the first week. Second, the increased rate of loss of I^{131} from the gland, previously described as a radiation effect, did not occur until the second week. Third, the calculated radiation delivered to the thyroid was not correlated with the presence or amount of triiodothyronine. On the other hand, patients with an increased rate of conversion of iodide to organic forms and with a high level of circulating thyroxine showed significant amounts of triiodothyronine in the serum (Table 3). These observations suggest that the presence of triiodo-

TABLE 3. CONVERSION OF RADIOIODIDE TO ORGANIC FORM

Per cent conversion in 24 hrs.	No. of patients			
	Total	Significant compounds in serum		
		Tri	Mono	Di
Below 20	6	0	1	1
20–40	5	2	4	2
41–80	5	3	2	1
Over 80	14	10	4	1
Totals	*30*	*15*	*11*	*5*

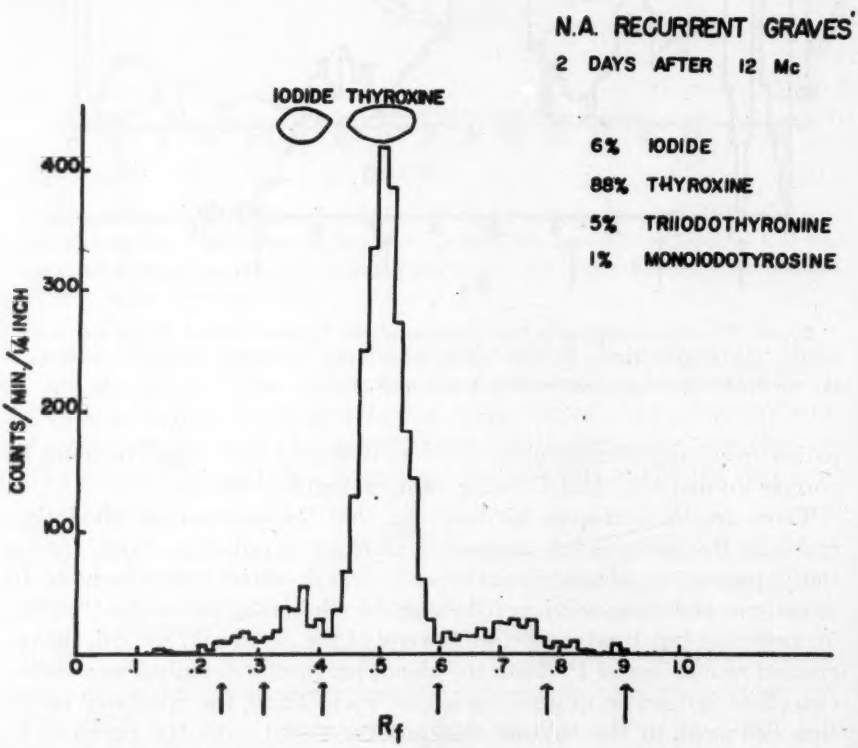

FIG. 5. This chromatograph shows the location of carrier amounts of unlabeled iodide and thyroxine added at the point of application. The spots were stained by palladium chloride and ninhydrin®, respectively.

thyronine was related to the degree of thyrotoxicosis and not to the effect of radiation on the gland.

Some monoiodotyrosine was found in the serum of 14 patients, and in 11 this amounted to more than a trace. The largest amount was 11 per cent, occurring on the fourth day in a patient who had received 8 millicuries of I^{131} for treatment of Graves' disease. Seven patients had monoiodotyrosine in the serum the first day, and in only 2 patients was it first observed after ten days. Most of the patients with detectable monoiodotyrosine responded well symptomatically to I^{131}, but some who received large amounts of radiation did not show detectable monoiodotyrosine in the serum.

Diiodotyrosine was identified in the serum of 6 patients who received large doses of radioiodine and in whom the radiation delivered to the thyroid exceeded 850 microcurie-days per estimated gram. Diiodotyrosine appeared in the serum during the first four days in all except 1 of these patients. The largest amount detected was 5 per cent, occurring the first day in the patient who received 35 millicuries to destroy a small fragment of normal gland remaining after operation for thyroid cancer (Fig. 6). Ingbar and collaborators (12) found only traces of diiodotyrosine when they chromatographed butanol extracts of serum after the administration of large doses of radioiodine, and they believed these traces were due to decomposition of thyroxine. Although breakdown of thyroxine could have occurred in the system used here, pure radiothyroxine[5] extracted from serum did not decompose to either monoiodotyrosine or diiodotyrosine during chromatographic fractionation.

DISCUSSION

The major problem in drawing conclusions from studies which are made following the administration of therapeutic amounts of radioiodine is to determine which results represent radiation effects, which represent disease processes, and which represent normal physiologic events. Final conclusions cannot be reached at this time but indirect evidence may permit some tentative interpretations.

The depression of the level of radioiodide which occurs on the first or second day following the administration of the I^{131} is probably due to the rapid clearance of the radioiodide from the blood. The reason for the subsequent increase in the amount of circulating iodide is another matter. This rise may result from beginning deiodination of the labeled thyroxine in the body after it has been secreted from the gland. Two of the patients showing this phenomenon had only a small loss of I^{131} from the gland at this time. This suggests that the effect was not due to radiation but that changes in

[5] Furnished through the courtesy of Dr. Edward O. Harper.

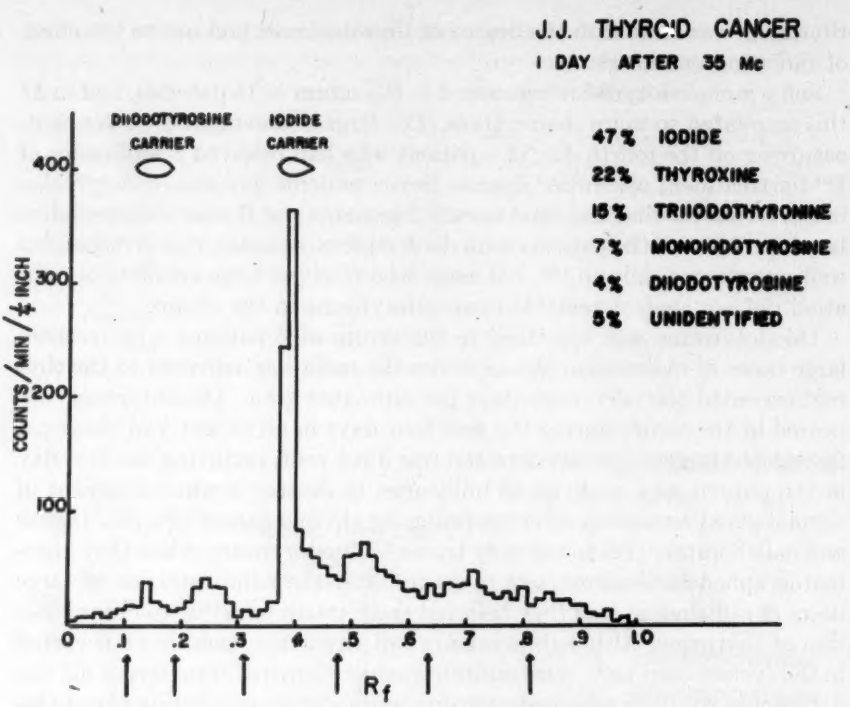

FIG. 6. This patient had an estimated gram of normal thyroid gland remaining following an operation for thyroid cancer. Note the location of the carrier compounds.

the specific activity of the iodide released from hormone in the peripheral tissues may be the cause of the subsequent increase of radioiodide in the circulation. In spite of arguments to the contrary, the possibility remains that this radioiodine was released from the thyroid by the radiation effect.

Although Gross and Pitt-Rivers (3) have found triiodothyronine in the plasma of a patient with Graves' disease who received only a tracer amount of radioiodine, most methods, including the one described here, require large doses of I^{131} to make possible the identification. Although a moderately large amount of I^{131} was needed for the identification, triiodothyronine was present in most of our patients soon after the administration of the treatment dose of I^{131} and before radiation damage would be expected to occur. If it be argued that there was sufficient radiation effect within the first twenty-four hours to produce triiodothyronine, then it would be expected that much more of this compound would be present some days later. An additional argument is that triiodothyronine was not detected in the serum of some patients with numerous manifestations of a radiation effect,

whereas it was detected in some patients who were inadequately treated. This evidence suggests that the occurrence of triiodothyronine in the serum is not necessarily an effect of radiation. The presence of triiodothyronine was correlated with the level of thyrotoxicosis as measured by the rate of conversion of iodide to the organic forms in the blood and by the amount of circulating radiothyroxine.

Monoiodotyrosine was recovered from the serum more often and in larger amounts than diiodotyrosine. This is in contrast to the findings reported by Robbins and coworkers (1). The presence of monoiodotyrosine could not be related to the evidences of radiation effect or to hyperfunction of the thyroid. Although it was found in 2 cancer patients with presumably normal thyroid remnants, it was also found in 12 patients with hyperthyroidism.

Diiodotyrosine, on the other hand, was noted in the serum of patients receiving the most radiation of the thyroid. It appeared promptly in the serum of 1 cancer patient who had a less than a gram (estimated) of normal tissue remaining after operation; in contrast, it was not found until twelve days after the administration of I^{131} in another patient who had an estimated 20 grams of normal gland. Thus it seems possible that the presence of diiodotyrosine in the serum is the result of irradiation of thyroid tissue. The quantities identified may be small, because only about 50 per cent is butanol-extractable and because of rapid destruction in the body (13, 14).

The presence of unknown compounds, such as those described by Gross and Leblond (2), has not been excluded by this study. In fact, occasional chromatograms showed unidentified radiation areas near the solvent front and between the iodide and thyroxine peaks.

SUMMARY

Iodinated compounds which appear in the serum after the administration of a therapeutic dose of I^{131} have been isolated by chromatographic fractionations, following 34 radioiodine treatments for hyperthyroidism or thyroid cancer. The relationships between the time of appearance of these compounds, the clinical response of the patient, the amount of radiation delivered to the thyroid, and the rate of disappearance of radioactivity from the gland have been discussed. Triiodothyronine was detected in the serum in 19 patients, monoiodotyrosine in 14 and diiodotyrosine in 6. Evidence is presented to suggest that the presence of triiodothyronine is related to the degree of thyrotoxicosis and not to the effect of radiation. The presence of diiodotyrosine appeared to be the result of radiation damage to the thyroid gland. Following radioiodine therapy, a change toward an increasing rate of loss of I^{131} from the thyroid may be an indication of radiation effect.

Acknowledgment

The authors wish to express their gratitude to Miss Nadine Berger and Miss Anne Ninmer for invaluable technical assistance.

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THE INFLUENCE OF VARIOUS FACTORS ON THE UPTAKE OF IODINE BY THE THYROID*

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THE mechanism of thyroidal concentration of iodide is unknown. A thyroid-iodide/serum-iodide concentration ratio of 25:1 occurs in normal rat thyroid and may increase to 200–300:1 during chronic administration of an antithyroid agent (1).

Various factors influence this iodide-concentrating mechanism. The quantity of iodine per 100 grams of thyroid tissue has been demonstrated to have an inverse relationship to the thyroidal iodide-concentrating ability (2), and a high concentration of serum iodide decreases the thyroid-serum iodide ratio (1). Hypophysectomy results in a reduction of the concentrating ability, which is restored by thyrotropin (3). This effect of thyrotropin seems to be enhanced by propylthiouracil, but the administration of propylthiouracil alone in the hypophysectomized animal has an inconstant effect on the iodide concentration gradient (4). The thyroid-serum iodide ratio in hypophysectomized animals may be lowered still further by the administration of thyroxine (4).

Potassium thiocyanate reduces the thyroidal accumulation of iodide (5). The action of some other monovalent anions (perchlorate, chlorate, hypochlorite, periodate, iodate, biiodate and nitrate) is similar (6). The effect of thiocyanate and perchlorate in interfering with thyroid collection of iodide may be overcome by added iodide. Schachner, Franklin and Chaikoff (7) demonstrated that the iodide-concentrating mechanism is independent of that for the organic binding of iodine. Sulfide and cyanide reduced the uptake of radioactive iodine in tissue slices, as well as its conversion to thyroxine and diiodotyrosine. Azide and sulfanilamide, however, almost completely suppressed the formation of thyroxine and diiodotyrosine but the iodide-concentrating capacity remained almost unaltered.

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† Damon Runyon Memorial Fund Fellow.

A study of the influence of other chemical and physical agents on the iodide-concentrating mechanism is presented.

MATERIAL AND METHODS

Fresh sheep thyroids were obtained at the local abattoir[1] and iced. Slices about 0.5 mm. thick were placed in a buffered medium containing propylthiouracil (10^{-3} M) to prevent organic binding of iodide, and allowed to stand fifteen minutes. The slices were blotted, weighed, and 100–150 mg. placed in tubes containing 1 or 2 cc. of medium to which 5 μc. of carrier-free I^{131} had been added. Test substances were added, to a final concentration of usually 10^{-3} M. A few experiments were performed in which air was replaced with nitrogen above the medium. The tubes were then placed in a constant temperature (37–38° C.) bath and shaken at 120 cycles per minute for one to two hours. Aliquots of 0.1 cc. were then removed, made up to 1-cc. volumes, and placed in 10-cc. flat-bottomed vials. Radioactivity of samples was determined by the method of Raben (8). Uptakes were similar with either Krebs-Ringer phosphate or bicarbonate media.

Effects on respiration of substances found to be blockers of iodide uptake were studied in a Warburg apparatus. Test materials were added directly to the medium, either at the beginning, or later from the side arm. In these studies the gaseous phase was air, and the medium was Krebs-Ringer phosphate medium with I^{131} and propylthiouracil. The experiments were continued for usually ninety minutes at constant temperature (37–38° C.) and then respiration (QO_2) was determined (wet weight).

The amount of I^{131} taken up by the tissue was determined by comparing aliquots from vessels containing no tissue with those from the test vessels. All experiments were controlled by several vessels containing tissue but without test chemical substances. Results were expressed as a percentage of the control QO_2 and a percentage of the control thyroid-medium iodide ratio.

RESULTS

The uptake of iodide was normally between 50–90 per cent, and the thyroid-medium iodide concentration ratio ranged from 10 to 130, usual values being 20 to 30.

Replacement of the atmosphere by nitrogen resulted in a decrease of the iodide concentration. Extremes of temperature (2° C. and 80° C.) and pH were inhibitory. At 2° C. the iodide concentration gradient approached 1. The ability of the slice to concentrate iodide was similar with media pH ranging between 6.6 and 8.6, but diminished at more extreme pH values. Mincing resulted in a fall in uptake of iodide and thin slices were more efficient than thick slices (Fig. 1). Physiologic sodium chloride seemed to be as effective as the phosphate or bicarbonate-buffered Ringer's. The addition of glucose and several amino acids (including glycine, cystine, cysteine, tyrosine, histidine and phenylalanine) had no effect on the thyroid-medium iodide ratio.

One hundred and eighty compounds were tested. The following are some

[1] Courtesy of Mr. Harold Larsen, New England Dressed Meat and Wool Company.

of the substances which had no effect, at 1×10^{-3} M concentration, on the thyroidal iodide concentration gradient: acetic acid, acetylcholine, alizarin Red S, allantoin, alloxan, aloin, *o*-aminobenzoic acid, *p*-aminosalicylic acid, aniline, anthraquinone, atropine, beryllium sulfate, boric acid, bromthymol blue, butazolidine sodium, catechol, chloramphenicol, chromium trioxide, cobalt chloride, congo red, cysteine, cystine, desoxypyridoxine, dichlorobenzene, dodecyl mercaptan, epinephrine, ergot, ethionine, flavianic acid, glycine, hemotoxylin, heparin, histamine, histidine monohydrochloride, *l*-histidine, hydrogen peroxide, insulin, lithium chloride, magnesium chloride, 2-methyl-3-phytyl-1,4-naphthoquinone (Mephyton), mercaptoacetic acid, naphthalene-β-sulfonic acid monohydrate, naphthoresor-

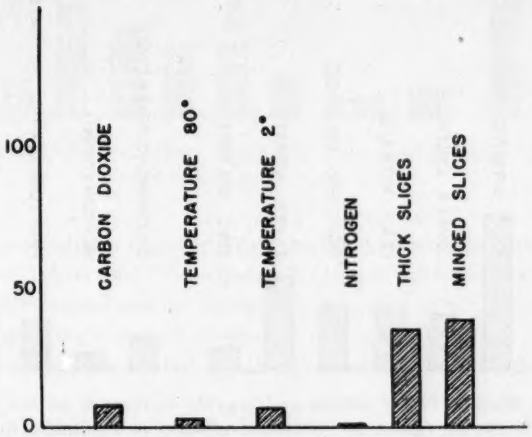

FIG. 1. The thyroid-medium iodide ratio expressed as a percentage of the control (considered as 100 per cent). Carbon dioxide caused a pH change which may account for its effect.

cinol, neutral red, para-aminobenzoic acid, pantoyltauryl anisidide, phenol, phenylalanine, phloroglucinol, physostigmine, potassium chloride, pyridine sulfonic acid, *p*-quinone, resorcinol, salicylamide, sodium azide, sodium bromate, sodium chloride, sodium diethylbarbiturate, sodium fluoride, sodium formate, sodium molybdate, sodium monobasic phosphate, sodium nitrite, sodium sulfide, stilbestrol, streptokinase-dornase, streptomycin, succinic acid, sulfanilamide, thiamine, tyrosine, ethylenediaminetetraäcetic acid (Versene), vitamin A, vitamin B_6 and xanthydrol.

Most compounds which reduced the iodide uptake react with sulfhydryl groups. The following substances lowered the thyroid-medium iodide ratio: heavy metals (arsenic trioxide, copper chloride, lead nitrate, mercuric chloride, silver nitrate and zinc sulfate); organic mercurials and halogen-

ated organic substances (chloromercuric benzoate, Mercuhydrin, phenylmercuric nitrate and mercaptomerin sodium); quinones (hydrolapachol, hydroquinone, menadione, quinhydrone, sodium anthraquinone-β-sulfonate and menadione diphosphate); alkylating agents (iodoacetate and bromoacetate); oxidizing agents (ferricyanide and nitroprusside); and organic dyes (methylene blue, rhodamine and eosin Y). Some other inhibitory compounds were Atabrine, bromsulphalein, hexylresorcinol, dinitrophenol, Dicumarol, Ninhydrin, penicillin, quinine and prophenpyridamine

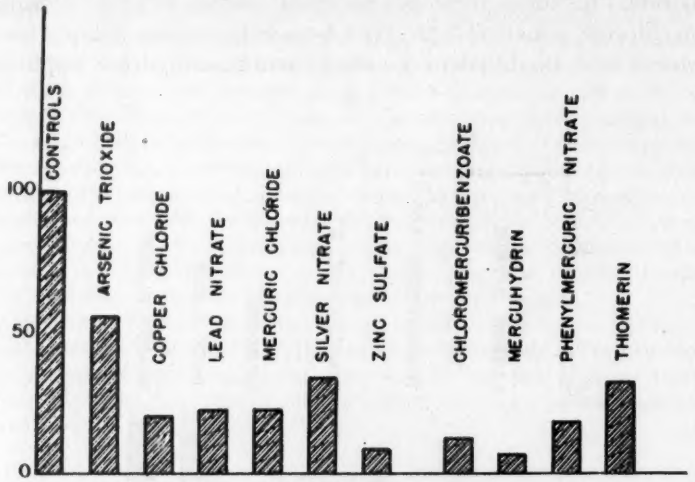

FIG. 2. The effect of heavy metals and organic mercurials on the thyroid-medium iodide ratio. Nitrate ion (given as potassium nitrate) in equimolar quantities did not cause the degree of reduction brought about by lead nitrate, silver nitrate and phenylmercuric nitrate.

(Trimeton) (Figs. 2, 3 and 4). Some substances were given as nitrates. Controls with equimolar amounts of potassium nitrate showed that the inhibitory action of the nitrate ion was too small to account for the fall in iodide gradient produced by these substances.

Some substances which inhibited the uptake of iodide were tested for ability to empty the slice of iodide. Certain substances which were effective as blockers were much less effective as emptiers (*e.g.*, Ninhydrin, menadione, dinitrophenol and arsenic trioxide), but many were capable of greatly reducing the concentration of iodide already present in the thyroid (*e.g.*, phenylmercuric nitrate, mercuric chloride, methylene blue and rhodamine) (Fig. 5).

As many inhibitors of iodide-concentrating ability react with sulfhydryl groups, cysteine was added in some instances. Cysteine effectively prevented the inhibiting action of certain substances (menadione, mercuric

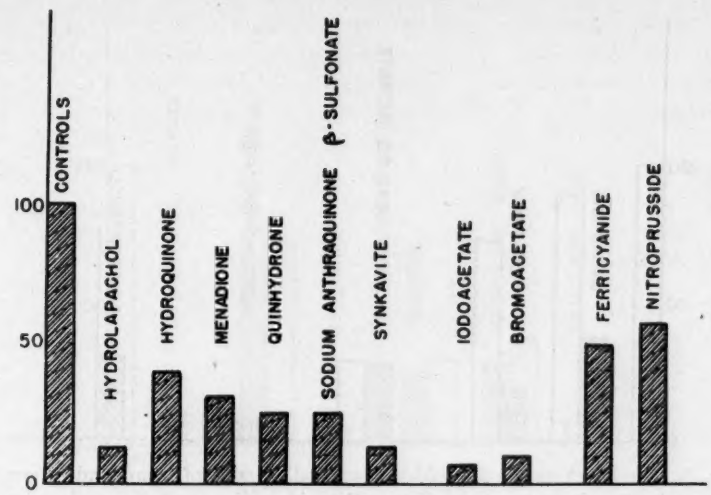

FIG. 3. The effect of the quinones, alkylating agents and oxidizing agents on the thyroid-medium iodide ratio.

chloride and menadione diphosphate), but not of others (methylene blue, rhodamine and Trimeton) (Figs. 6 and 7). It is of interest that the addition of cysteine and menadione in equimolar quantities occasionally resulted in an iodide uptake greater than that of the controls.

Of the 40 compounds tested by the Warburg direct method, many re-

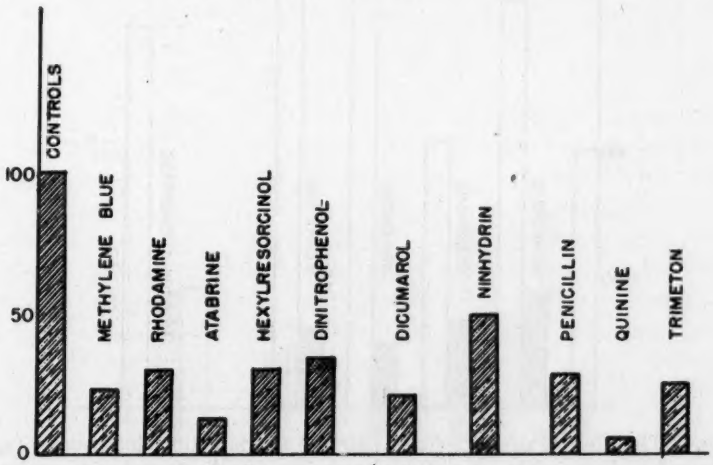

FIG. 4. The effect of organic dyes and other compounds on the thyroid-medium iodide ratio.

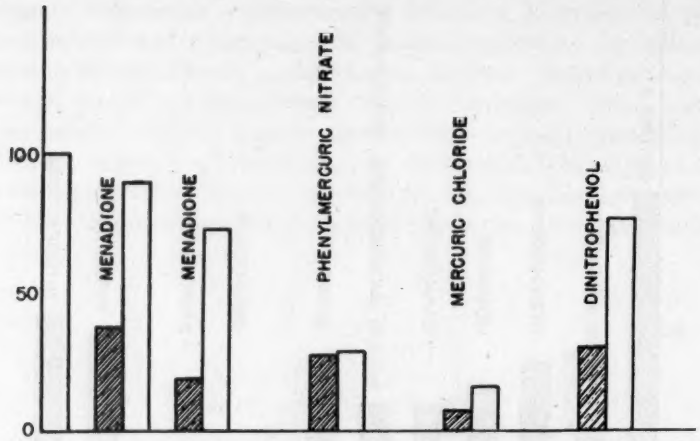

FIG. 5. The effect on the thyroid-medium iodide ratio of compounds given before (cross hatched) and after (open) iodide uptake. Menadione and dinitrophenol are relatively ineffective in emptying iodide already concentrated in the slices.

vealed a proportionate decrease in QO_2 and iodide uptake (bromsulphalein, Dicumarol, dinitrophenol, eosin Y, cyanide, hydroxylamine, mercuric chloride, quinine and quinidine) (Fig. 8); but some diminished the iodide uptake with comparatively little or no diminution in QO_2 (potassium thio-

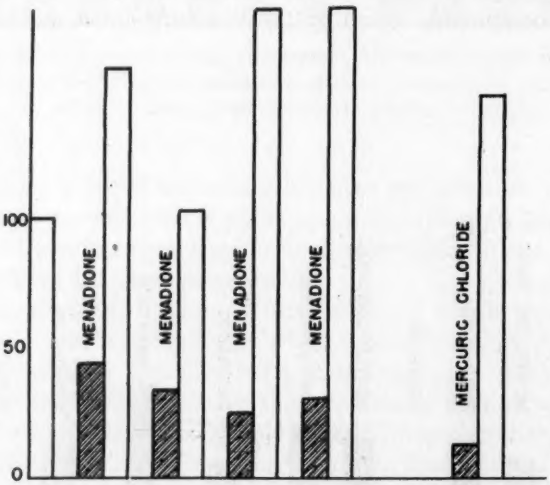

FIG. 6. The effect of inhibitor (cross hatched) and inhibitor plus cysteine (open) on the thyroid-medium iodide ratio. Several experiments indicate an iodide concentration gradient above normal.

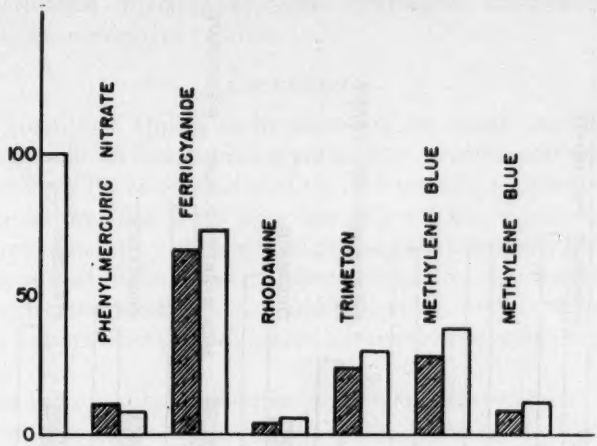

FIG. 7. The effect of inhibitor (cross hatched) and inhibitor plus cysteine (open) on the thyroid-medium iodide ratio. The addition of cysteine did not restore the iodide concentrating mechanism.

cyanate, silver nitrate, Mercuhydrin, phenylmercuric nitrate, mercaptomerin sodium, sodium anthraquinone-β-sulfonate, iodoacetate and Ninhydrin) (Fig. 9). When methylene blue was added to cyanide-inhibited slices, the respiration was restored out of proportion to the increase in iodide concentration gradient (Fig. 10). Certain experiments suggested that an increase above the normal QO_2 and iodide concentration gradient occurred when cysteine was added with menadione.

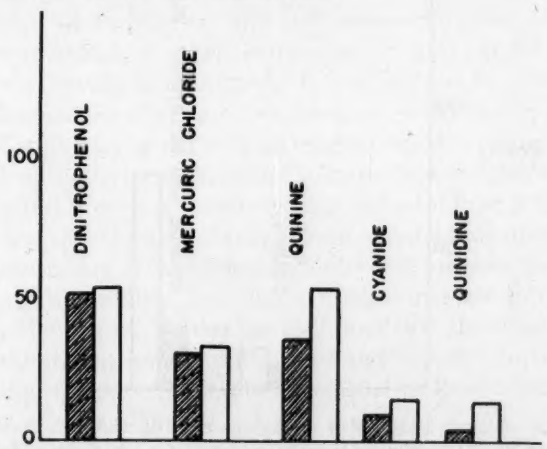

FIG. 8. The effect on iodide concentration gradient (cross hatched) and QO_2 (open) of some inhibitors which produce a proportional decrease in each.

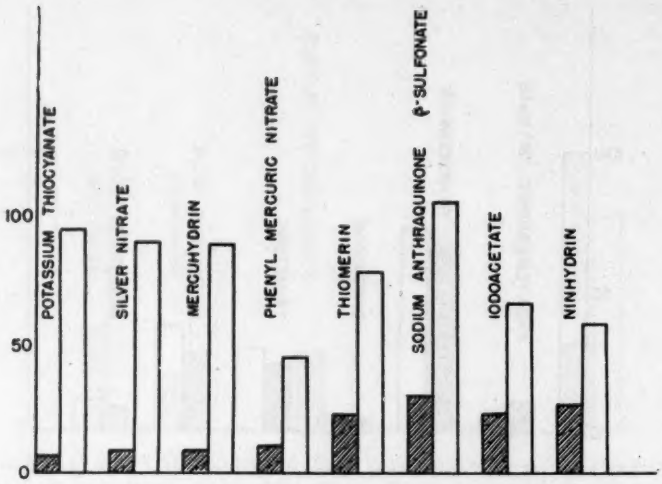

FIG. 9. The effect of iodide concentration gradient (cross hatched) and QO_2 (open) of some inhibitors which reduce the ability to concentrate iodide more than the respiration of the thyroid slices.

Attempts to produce goiter in rats by feeding some of these compounds admixed with a low-iodine diet were unsuccessful, often because of toxicity. Acute experiments designed to change the thyroid-serum iodide ratio

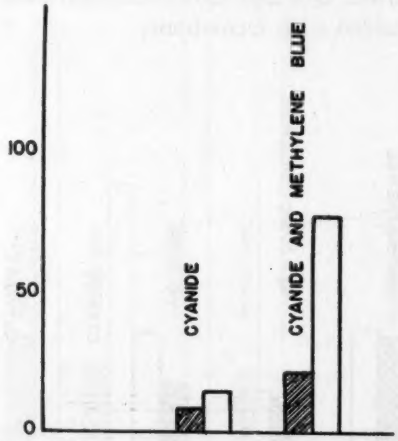

FIG. 10. The effect of addition of methylene blue on the cyanide-inhibited iodide-concentrating ability (cross hatched) and respiration (open) of thyroid slices. There is a greater restoration of respiration than of iodide concentration. Methylene blue acts as an electron acceptor in the cyanide-inhibited enzyme systems.

by intraperitoneal injection of these compounds in propylthiouracil-treated rats gave negative results.

DISCUSSION

Aerobic conditions appear to be necessary for iodide accumulation as shown by the reduced concentration gradient in an atmosphere of nitrogen. Marine and Feiss (9) also found that thyroid must be respiring in order to concentrate iodine. The thick slice has a lower concentration gradient, probably due to inability of oxygen to diffuse into the center of the slice.

Both tissue metabolism and iodide-concentrating mechanism are impaired by extremes of temperature and pH, yet a simple medium such as physiologic sodium chloride can be used without lowering iodide concentration.

It has not been possible to depress tissue respiration without depressing the iodide uptake. There are, however, several instances of inhibited iodide concentration with approximately normal respiration. Lundegårdh (10) has expressed a theory of "anion respiration," describing a close relationship between the active transport and accumulation of electrolytes intracellularly and the transference of electrons and protons from the dehydrogenases through the cytochrome system to oxygen; the cytochrome system must be complete in order to accumulate anions but cytochrome respiration will continue in spite of blocking, say, cytochrome B. There may, then, be normal tissue respiration with a loss of ability to accumulate anions.

The importance of sulfhydryl groups for iodide trapping is apparent, as many sulfhydryl group inhibitors repress the iodide concentration gradient. As over 50 per cent of respiratory enzymes are sulfhydryl-containing, the relationship of respiration and iodide-concentrating ability is suggested. The importance of other nonrespiratory sulfhydryl-containing enzymes, however, should be considered. It is of interest to note that Barron (11) has indicated that there are two kinds of sulfhydryl groups—the so-called soluble sulfhydryl groups which tend to regulate cellular respiration, and the fixed sulfhydryl groups bound to important cellular enzymes. The soluble sulfhydryl groups regulate respiration by inhibiting the rate of reoxidation of the cytochrome system. There is some indication that a certain low concentration of sulfhydryl inhibitor will increase respiration and iodide-concentrating ability, but that a further increase in concentration will inhibit both of these. Barron has indicated that the soluble sulfhydryl groups are inhibited at low concentrations and that the important sulfhydryl-containing enzymes are inhibited at higher concentrations.

The failure of azide, fluoride, alloxan, and other compounds to inhibit the iodide-concentrating mechanism and respiration may be due to the complexity of the system studied; denatured proteins present may nullify

their effect (12), secondary enzyme pathways may substitute for an inhibited pathway, and the passage of the material across the cell membrane may be limited. The concentration of the substance tested and the pH of the medium may determine whether or not inhibition takes place. Dinitrophenol, for example, is a definite inhibitor of respiration and iodide-concentrating ability; at lower concentration, however, respiration may be stimulated. Naphthoquinones are much more active on the succinic oxidase system at pH 7.0 than at pH 8.0 (13). The toxicity of iodoacetate for yeast cells occurs only at acid pH (14).

The failure of certain substances, capable of inhibiting the uptake of iodide ions, to empty from a slice the already accumulated iodide could imply the binding of the iodide by the inhibitor outside the cell. This seems most unlikely chemically. The physiologic requirements for anion entrance into the cell may not be essential for anion retention in the cell.

There is a possibility that these results apply to the transfer of other electrolytes across cell membranes. There is no evidence that the effects on the iodide-trapping mechanism observed in this study are restricted to iodide.

SUMMARY

The iodide concentration gradient and QO_2 have been determined in fresh sheep thyroid slices. Aerobic conditions were necessary for iodide concentration. Extremes of temperature and pH diminished the ability of the thyroid slice to accumulate iodide. Many compounds which react with sulfhydryl groups were found to diminish the iodide gradient. An inhibitor of iodide uptake may or may not empty out iodide already concentrated. Compounds which depress the iodide-concentrating capacity do not necessarily depress the respiration of the slice, though most of them do. A factor which increases slice respiration may increase the iodide concentration but no substance has been found to depress respiration without also depressing the iodide-concentrating mechanism.

Acknowledgment

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Teaching Clinic

DIFFERENTIAL DIAGNOSIS OF VIRILISM

THE differential diagnosis of virilism is frequently difficult. The problem may be complicated by lack of understanding of the exact definition of virilism. For practical purposes, it may be defined as an abnormal endocrine state characterized by signs of excessive production of androgen. This abnormal state can best be illustrated in a woman who received testosterone propionate therapy for advanced mammary cancer.

Case No. 1

Mrs. B. B. (OC No. 73294), a white woman, aged 54 years, had inoperable mammary carcinoma. She had had her last menstrual period at the age of 44 years. She was treated with intramuscular injections of 100 mg. of testosterone propionate in oil three times weekly. Within a few weeks hirsutism developed over the face, body and abdomen. Her voice became slightly deeper, acne appeared over the face and shoulders, and there was increased oiliness and coarseness of the skin generally over the body. At the time of examination six months later, an increase in the size of the clitoris was noted. Development of male musculature, loss of some of the feminine body fat, and greatly increased libido occurred during this period of androgen therapy.

This case illustrates the more prominent signs and symptoms associated with the onset of virilism, whether produced by endogenous or by exogenous means. In many women who have not yet undergone the menopause, amenorrhea and atrophy of the breasts are frequently seen during androgen therapy. Amenorrhea develops shortly after therapy has been instituted. The urinary 17-ketosteroid excretion increased approximately 8 mg. per twenty-four hours in a group of patients with advanced cancer of the breast while receiving daily intramuscular injections of 50 mg. of testosterone propionate. The increased libido associated with testosterone therapy does not often occur in virilism of endogenous origin. To our knowledge, there have been reports of only 2 patients with androgenic lesions of the adrenal associated with hirsutism as the only sign of androgen production. Most patients have the assorted signs and symptoms which would be expected when an excessive amount of androgen is present (1, 2). It should be added that the 2 patients mentioned, who had hirsutism without true virilism and in whom androgenic lesions of the adrenal were found, showed increased urinary 17-ketosteroid excretion. Therefore, the measurement of the urinary 17-ketosteroid excretion might be of distinct value in finding

the occasional rare androgenic tumor producing hirsutism as the only clinical sign.

The next case illustrates idiopathic hirsutism without true virilism.

Case No. 2

Miss C. C. (*OC No. 30728*), a white girl, aged 16 years, had noted slight hair growth on the upper lip for several years. In the previous year, the hair growth of the lip had increased and hirsutism developed over the arms, breasts and lower abdomen. The facial hair required shaving at least once a week. There was a history of similar hair distribution in other female members of her mother's family. The patient had regular menses, normal mammary development, a feminine voice, and a clitoris of normal size. The urinary 17-ketosteroid excretion was 13.1 mg. per twenty-four hours.

This patient was believed to have simple hirsutism, not virilism. This is by far the most common type of case in which virilism is suspected by either the family or the physician. Since it is usually familial, being common in women whose ancestors lived in Mediterranean areas, and unassociated with increased urinary 17-ketosteroid excretion, it is probably a genetic disease in which the hair follicles are apparently overly sensitive to the amount of androgen usually present in the female. It can best be called nonendocrine heterosexual hypertrichosis.

The third example is a patient with an ovarian hilus-cell tumor as the source of the excessive androgen.

Case No. 3

Mrs. B. M. (*OC No. 94088*), a white woman aged 39 years, suddenly stopped menstruating at the age of 33 years, without any associated menopausal symptoms. One year later hair began to grow on the face and over the back of the thighs and lower abdomen, and the hairline began to recede at the forehead. Her singing voice changed from soprano to baritone. Her skin became roughened and reddish in color, and acne and mild dermatitis developed. Libido was not increased. The musculature over the body and about the neck was strikingly thickened. The only pelvic abnormality was a clitoris twice normal size. The urinary 17-ketosteroid excretion on three repeated determinations averaged 15.6 mg. per twenty-four hours. A biopsy specimen of the endometrium showed atrophic and cystic changes. In excretory urograms of the kidneys no abnormalities were evident. Abdominal exploration revealed a right ovarian tumor, which was removed and found, upon pathologic examination, to be a hilus-cell tumor.

The lesion in such a patient may be a localized hilus-cell tumor or hyperplasia of the hilus cells (3). Though generally considered an unusual cause of virilism, this ovarian lesion has been one of the most common encountered by us; less common are arrhenoblastomas and adrenal-cell rests.

The most characteristic features in Case 3 were the development of true virilism, without a marked increase in urinary 17-ketosteroid excretion. Although the urinary 17-ketosteroid excretion was not considered to be elevated preoperatively, postoperatively the values were 11.6 and 9 mg.

per twenty-four hours. This drop in urinary 17-ketosteroid excretion following removal of a hilus-cell tumor is a rather typical picture and indicates that the hilus-cell tumor was contributing to the urinary 17-ketosteroid excretion in this patient. Of help in the differential diagnosis was the normal pyelogram, although this may also be noted in cases of adrenal hyperplasia or small adrenal tumors.

Ovarian lesions without the complete clinical picture of virilism, may cause some confusion in diagnosis. Such an example is the next case.

Case No. 4

Miss D. G. (*OC No. 144672*), a white woman, aged 27 years, had not menstruated since she was 18. The menarche occurred when she was 12 years old and she had normal menstrual periods until the age of 18 years. The only menses since that time followed cyclic hormonal therapy. Facial and body hirsutism had developed in the previous five years, to such a degree as to require daily shaving. Her weight had gradually increased from 120 to 140 pounds during this period. She had not noticed deepening of the voice, acne or increased oiliness of the skin. Purple striae were noted on the abdomen. The breasts were normally developed, the clitoris and cervix were small, and the ovaries were not enlarged. Excretory and retrograde pyelograms, an aortogram, and perirenal air studies showed a normally placed kidney without any visible adrenal tumor. The 24-hour urinary excretion of 17-ketosteroids was 14 mg., and of formaldehydogenic corticoids, 0.74 mg.; there was no pregnanediol chromogen. Exploratory laparotomy revealed small adrenals and polycystic ovaries twice normal size, typical of the Stein-Leventhal syndrome (4). Operation consisted of wedge resection and suspension of both ovaries. The microscopic picture of severe ovarian cortical stromal hyperplasia, and abundant follicular development with cystic and atretic follicles associated with fibrosis and thickening of the cortex, was in agreement with the Stein-Leventhal type of ovary. Of interest was the fact that the patient began to menstruate four days postoperatively.

This case was very instructive, in that the history and results of physical examination led us to believe that the patient probably had a mixed adrenal tumor. However, the normal urinary hormone excretion tended to rule out that possibility. The presence of hirsutism and amenorrhea associated with normal urinary 17-ketosteroid excretion further pointed towards an ovarian lesion, although no ovarian tumor could be palpated on pelvic examination.

The adrenal cortex may also be the site of conditions leading to the development of virilism. Increased production of androgen may result from either hyperplasia or a tumor of the cortex, as illustrated by the following 2 cases:

Case No. 5

Miss J. M. (*OC No. 139809*), a 17-year-old white woman, complained of primary amenorrhea. Body hair appeared at the age of 8 years and deepening of the voice at the age of 11 years. The breasts had never developed. Physical examination showed generalized body hirsutism, atrophic breasts, and an enlarged clitoris approximately 1½ inches in length. Only rudimentary uterine tissue could be felt on rectal examination,

and no definite ovarian tissue could be palpated. Excretory urograms gave normal findings and neither kidney seemed to be displaced. The 24-hour urinary excretions of 17-ketosteroids and pregnanediol chromogen were 163 mg. and 152 mg., respectively. The urinary dehydroisoandrosterone test gave a negative result. Upon intravenous administration of compound F the urinary 17-ketosteroid excretion decreased to 8 mg. per twenty-four hours, and the pregnanediol chromogen excretion fell to zero. Intramuscular injection of cortisone acetate resulted in a clinical remission and sustained suppression of the excessive steroid excretion (5).

This patient undoubtedly had bilateral adrenal hyperplasia which had become manifest clinically in early childhood, with the development of virilism at that time. The elevated urinary 17-ketosteroid and pregnanediol chromogen excretions suggested an adrenal lesion. In addition, the decrease in steroid excretion following intravenous injection of compound F suggested adrenal hyperplasia rather than adrenal tumor (6). Since cortisone therapy resulted in menstruation, mammary development, and regression of the acne and hirsutism to some degree, the diagnosis of adrenal hyperplasia appeared to be definite.

Case No. 6

Mrs. M. M. (*OC No. 137448*), a 32-year-old white woman, had generalized hirsutism of eight months' duration. Although the hair that had been previously present had become longer and darker, and new hair had developed upon the face, breast, abdomen and legs, the hair on her head had begun to fall out. She noticed deepening of the voice, increased oiliness of the skin, acne, and decrease in libido. There had been no menses for the previous five months. Fatigue and loss of weight were prominent. On pelvic examination, there was a slightly hypertrophied clitoris and a somewhat enlarged left ovary. An excretory urogram showed a slightly ptotic right kidney. The urinary 17-ketosteroid excretion varied from 71 to 97 mg. per twenty-four hours, and the pregnanediol chromogen excretion averaged 6.3 mg. per twenty-four hours. The urinary dehydroisoandrosterone test gave a negative result. After one month of cortisone therapy, 50 mg. intramuscularly daily, the urinary 17-ketosteroid excretion was 60 mg. per twenty-four hours, which was not considered to be a significant decrease from the pretreatment level. On abdominal exploration, normal ovaries were found. A right adrenal tumor was removed by Dr. Rawley M. Penick, Jr. Postoperatively, the urinary 17-ketosteroid excretion was 4.6 and 4.9 mg. per twenty-four hours. After operation the menses were regular, and there was a remarkable remission of the acne and cutaneous changes.

This last case of adrenal tumor with virilism and an elevated 17-ketosteroid excretion which did not decrease as a result of cortisone therapy, emphasizes one of the differences between patients with adrenal tumor and those with adrenal hyperplasia. It is of interest that the dehydroisoandrosterone test (7), which is generally considered to be more likely to yield a positive result in adrenal tumors, was of no help in the differential diagnosis in this case. In fact, the negative result was misleading. The displacement of the kidney noted in the urogram was of help in determining the diagnosis. In our hands, results of perirenal air insufflation studies have been

difficult to interpret in obese patients and have sometimes been misleading. Although we have used aortography infrequently, in one instance it was of great value in locating a pheochromocytoma; therefore, we believe this procedure may be worthwhile in the differential diagnosis of virilizing lesions.

Virilism can usually be differentiated from sexual precocity, because the latter always progresses along isosexual lines. Development of an androgen-producing lesion in a young boy or a feminizing lesion in a young girl could lead to isosexual precocity and, although extremely unusual, should be kept in mind.

SUMMARY

Primary emphasis should be placed on differentiation between simple hirsutism and true virilism. The latter shows all or most of the changes expected with excessive androgen production. A combination of hirsutism, obesity and amenorrhea without other evidence of virilism may be produced by the Stein-Leventhal type of ovarian lesion. The differential diagnosis can be facilitated by a careful history, physical examination and determination of urinary 17-ketosteroid and pregnanediol chromogen excretions. If the virilism is associated with a normal or questionably elevated urinary 17-ketosteroid excretion, it is most likely due to an ovarian lesion. In patients with elevated urinary 17-ketosteroids and pregnanediol chromogens, intravenous administration of compound F is the quickest means of determining an effect upon these excretions (6). A decrease in the levels of urinary 17-ketosteroids and pregnanediol chromogens following a 24-hour infusion is strong evidence in favor of a diagnosis of adrenal hyperplasia. In patients with adrenal tumors or ovarian lesions, on the other hand, the urinary 17-ketosteroid and pregnanediol chromogen levels do not change to any marked degree following infusion of compound F. The importance of differentiating the various types of virilism is emphasized by the fact that, whereas excellent medical therapy is available for virilizing adrenal hyperplasia, tumors are a surgical problem, and if amenable to curative extirpation must be detected as early as possible.

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Editorial

RESPONSE TO THYROID

WHEN desiccated thyroid is administered orally to myxedematous humans, the resultant metabolic changes are quite different from those observed in the euthyroid subject. The clinician can diagnose or treat hypothyroid patients much more intelligently if he is aware of these differences in response to thyroid. In addition, an unworked field for clinical research becomes evident when one reviews the known major differences between the myxedematous patient and the euthyroid subject in response to desiccated thyroid administration.

The most striking difference observed may be summarized by the statement that the patient who is truly deficient in circulating thyroid hormone responds dramatically and with predictable regularity to the administration of small doses of desiccated thyroid. The euthyroid subject, on the other hand, shows no obvious clinical response to small doses of thyroid. A simple and readily available diagnostic test for borderline hypothyroidism, therefore, is the administration of 1 grain of desiccated thyroid per day for a period of six weeks after a careful history and physical examination have been made and basal metabolic rate and serum cholesterol determinations have been performed. The patient who is truly deficient in thyroid hormone will generally respond to the administration of 1 grain of desiccated thyroid per day for six weeks with an increase in basal pulse rate and metabolic rate and a fall in the serum cholesterol level. The patient not truly deficient in thyroid hormone will show no significant change in these indices of thyroid hormone effect.

Means has used a therapeutic rough rule-of-thumb to demonstrate that a person severely deficient in thyroid hormone is more sensitive to exogenous thyroid than a person less deficient in thyroid. He has observed that ½ grain of U.S.P. thyroid per day will raise the BMR of a myxedematous subject from −40 per cent to −20 per cent. Doubling this dosage will raise the BMR further only to −10 per cent (1). The observations of Winkler and his co-workers (2) are not in exact conformity with this findings, but do show that the myxedematous patient is not only more sensitive to desiccated thyroid adminstration than is the euthyroid subject, but his response to desiccated thyroid is regular and predictable. Myxedematous patients were found to experience a rise in the serum precipitable-iodine level of 2.0

micrograms per 100 ml. and in the basal metabolic rate of 12 to 16 points for each grain of desiccated thyroid administered per day, in the dosage range up to 2 grains per day. The standard deviation in the serum precipitable iodine values was only ± 0.5 microgram and the standard error of the mean was ± 0.13 microgram per 100 ml. A euthyroid subject, on the other hand, rarely shows any change in these indices on doses up to 2 grains of desiccated thyroid per day (3). When any response is observed in the euthyroid subject it is temporary, whereas in the myxedematous patient these changes persist as long as the desiccated thyroid is administered. These differences between the myxedematous and the euthyroid subject, in response to small doses of desiccated thyroid, have been shown to be due to the presence of a normal thyroid gland in the euthyroid subject. The administration of exogenous thyroid induces a state of hypofunction in his thyroid gland, presumably by the mechanism of depressing the output of thyroid-stimulating hormone from the pituitary. This decrease in function of the patient's own thyroid prevents the added thyroid hormone from causing an increase in the patient's general body metabolism.

The myxedematous patient and the euthyroid subject also show a different response to sudden cessation of desiccated thyroid medication. The myxedematous patient at first shows a rapid drop in his serum precipitable-iodine level and basal metabolic rate, then a slower fall to pre-treatment levels over a period of six to eight weeks (3). On the other hand, when the thyroid medication of a euthyroid person is suddenly stopped, the serum precipitable-iodine value falls to myxedematous levels in three to four weeks, but the basal metabolic rate may take as long as nine to fifteen weeks to reach its lowest level (3). These values then rise to pre-treatment levels. The radioiodine uptake is also falsely depressed by thyroid administration and has taken as long as eleven weeks to recover (4). Furthermore, when the thyroid does recover its ability to concentrate I^{131}, the I^{131} pickup rate may rebound temporarily to hyperthyroid levels. Obviously, if a physician were to stop the desiccated thyroid medication of a euthyroid patient in an attempt to evaluate the patient's underlying thyroid status, these ensuing changes in indices of thyroid function, when sampled, might prove very misleading and confusing.

The daily output of thyroid hormone by the normal thyroid gland is thought to be equivalent to not more than 3 grains of desiccated thyroid per day. Consequently, when the daily dosage of desiccated thyroid is raised to 4 grains or more per day, any differences observed between athyreotic and euthyroid subjects can not be explained on the basis of further suppression of normal thyroid function. Most practicing physicians have probably observed a euthyroid patient who was comfortable while voluntarily taking 5 to 20 grains of desiccated thyroid a day, in an attempt to reduce

his weight and rid himself of fatigue. Rarely, if ever, does one see an athyreotic patient voluntarily take more than 3 grains of desiccated thyroid per day. This situation suggests that the athyreotic patient is more sensitive to thyroid substance than is the euthyroid person, even after his myxedema has been controlled with medication. Controlled observations have been made on euthyroid subjects given large doses of desiccated thyroid. Basal metabolic rates and serum precipitable-iodine values are not definitely and consistently maintained at elevated levels until the dosage of thyroid is increased to 10 grains per day or more (3). In the euthyroid person a tolerance tends to develop even to these doses, in periods of four to six months (5), as manifested by decreasing pulse rate, basal metabolic rate and concentration of serum precipitable-iodine. Thompson *et al.* (6) have published careful observations showing how myxedematous subjects handle single large doses of desiccated thyroid, and have compared single doses of many different thyroidal iodinated compounds in myxedematous and euthyroid subjects. They noted that, "A given dose of thyroxine has much less effect when themetabolism is normal before administration than when it is markedly reduced (minus 40 per cent)." We find no such careful observations of the effect of excessive doses of desiccated thyroid administered daily to myxedematous persons over a long period of time. If the treated myxedematous patient is more sensitive to large doses of thyroid, the even more interesting question is "Why is he?" No well substantiated explanation is apparent in the literature, although many interesting suggestions have been offered.

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Letters to the Editor

EFFECTS OF INTRAVENOUS PROGESTERONE ON THE CYTOLOGIC AND HISTOLOGIC CHARACTERISTICS OF HUMAN CERVICAL CARCINOMA*

To the Editor:

In a previously reported study (1) of the effects of intravenous progesterone on cervical carcinoma in women, the suggestion was put forth that the lack of a clear-cut demonstration that such treatment halted or changed the growth characteristics of the cancer could be due to the fact that all the patients selected for this study had previously been treated to the point of tolerance with radiation therapy. There is some evidence that radiation, in addition to its cancerostatic or cancerocidal effect, has a sclerosing effect on blood vessels (2), possibly particularly marked in such tumor tissue. The possibility could thus be entertained that the inadequacy of the blood supply to the cancerous tissue in this series of women played an important role in the failure of maximal effectiveness of the progesterone.

We felt, therefore, that a more appropriate way in which to study the effect of progesterone on cervical carcinoma would be to administer it to patients who had been previously untreated in any way. However, in order to achieve this objective without increasing the danger to the patient of delaying the institution of more accepted forms of therapy, we resolved to use a treatment period for these patients no more than ten days in length, and a post-treatment follow-up period of equal length, and to restrict our observations to the cytologic and histologic changes occurring in the cervix within this period of time. The study, thus, is not really one of the therapeutic value of progesterone, for obvious reasons; it is nothing more than an attempt to see whether progesterone affects previously untreated epidermoid cancer of the cervix in any way.

METHODS

A series of 5 women was selected for these observations. In 2 of them the diagnosis was cervical carcinoma Stage I, on the basis of clinical evalua-

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tion and the histologic characteristics of the pre-progesterone biopsy specimens; in the other 3, the carcinoma was classified as Stage III, on a similar basis. Biopsies were performed on all patients at least once before starting progesterone, then midway through the course of treatment, at the end of treatment, and at least once more sometime during the ten days following treatment. An attempt was made to obtain daily spreads from the cervix for staining by the Papanicalaou technique during the course of treatment; and then every other day in the ten days that followed treatment. This was not always successful, but at least enough smears were made for adequate evaluation.

All patients were hospitalized during treatment, and the progesterone[1] administered intravenously over a 12-hour or 24-hour period each day for ten successive days. The technique used for preparing the solutions has been described before (1, 3). The daily dose used in 4 cases was 500 mg., and in 1 case, 1,000 mg. In the latter case, however, the infusion had to be stopped after the eighth day, since the patient was beginning to show signs of renal failure.[2]

RESULTS

The results may be described quite simply. The 2 carcinomas of the cervix originally classified as League of Nations Stage I, were, on review of the slides, more correctly diagnosed as carcinoma *in situ*. In both of these, no visible change could be detected in the histologic characteristics of the carcinoma during or after the course of progesterone therapy. The cervical spreads, however, clearly showed a complete disappearance of malignant cells during the course of treatment, and a return of malignant cells during the post-progesterone period. In both cases, the disappearance was observed within the first five days of treatment, and the first sign of reappearance within a week following the last administration of the hormone.

The 3 carcinomas which were in a more advanced state at the time of diagnosis underwent no demonstrable change, as judged by the biopsy and spread evidences of cancer, at any time during or after the course of treatment with progesterone. There was some suggestion of increased inflammation in 1 of this group; however, this could not necessarily be ascribed to the progesterone.

[1] We are indebted to Dr. Edward Henderson, of Schering Corporation, Bloomfield, New Jersey, and to the American Red Cross, for the generous supplies of progesterone and human serum albumin, respectively, used in this study.

[2] These consisted of facial, pedal and pretibial edema, oliguria and albuminuria. Although it cannot be proved, we are fairly sure that they were attributable to the large amounts of albumin necessary to dissolve this dose of progesterone (450 cc. of 25 per cent human serum albumin) rather than to the progesterone itself. These signs regressed rapidly after the cessation of treatment.

Clearly these results mean only that, in cervical cells from an area of carcinoma *in situ*, the tendency to desquamate is decreased under the influence of large doses of progesterone, whereas in cells from a more advanced cancer, (*i.e.*, one that is already invasive) desquamation continues under the influence of progesterone. No further conclusion can be derived; in view of the fact that the biopsy specimens of the cervical carcinomas *in situ* did not show any definable histologic change.

So little is known about the essential nature of cervical carcinoma that, small though this effect is, it has seemed worthwhile to report it.

It is possible that the finding reported here is entirely adventitious. Hertz and Cromer (4) in reviewing their original findings (5) on the effect of progesterone on cervical carcinoma recently came to the conclusion that the hormone had no effect, the previously reported results presumably being due to spontaneous variations in the gross appearance and histologic characteristics of the tumor. This may well be so; but until more unquestionably negative evidence is presented, we are encouraged to continue the study of the impact of massive doses of intravenous progesterone on the cytology of cervical carcinoma. Additional support comes from Bradbury's (6) finding of a definite disappearance (for a period of three to four months) of malignant cells from the spreads of a patient with cervical carcinoma *in situ* treated with two short-term courses of intravaginal progesterone.

SUMMARY

In 2 patients with cervical carcinoma *in situ*, malignant cells disappeared from the cervical spreads, but there was no change in the histologic appearance of the biopsy specimen during a ten-day course of treatment with intravenous progesterone (500 mg. daily). In 3 other patients in a more advanced stage of the disease there was no change in either the cervical spread or the biopsy characteristics during or after the same or more intensive treatment with intravenous progesterone.

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PREGNANCY FOLLOWING SURGICAL REMOVAL OF A MASCULINIZING ADRENOCORTICAL TUMOR

To the Editor:

We wish to report a normal pregnancy occurring four and a half years after the successful surgical removal of a masculinizing adrenocortical tumor. Pregnancy following surgical extirpation of these tumors is rare. We were able to find reports of only 3 previous cases in the literature (1, 2, 3). Our case has been reported elsewhere prior to the pregnancy, and details of the earlier course may be found in that report (4).

CASE REPORT

A. B., a white female, was originally admitted to the Research and Educational Hospital of the University of Illinois in July 1948, at the age of 17 years, with a two-year history of amenorrhea and excessive growth of coarse black hair on the face, arms, buttocks, legs and abdomen (Fig. 1A). Urinary excretion of 17-ketosteroids was markedly increased, averaging 90 mg. per twenty-four hours. An intravenous pyelogram showed depression of the right kidney pelvis, and by perirenal insufflation a well outlined mass was seen in the region of the right adrenal gland. In August 1948, a well encapsulated adrenocortical tumor was removed. The patient made a good recovery. One month following operation, the patient had her first menstrual period in two years. Thereafter, she had regular periods every twenty-eight days, until the time of her pregnancy four and a half years later. Postoperatively, her urinary 17-ketosteroid excretion fell to 6.8 mg. per twenty-four hours. For several months after operation the patient noted a decrease in her hirsutism. Some facial hair persisted, however, making it necessary for her to shave (Fig. 1B). Pregnancy was diagnosed in March 1953, the last menstrual period having occurred on January 24. No abnormalities were noted during the pregnancy, and on November 8, 1953, the patient was delivered at full term, after a normal labor, of a male infant weighing 2,500 grams. The postnatal course was uneventful.

FIG. 1. **A:** Patient in 1948, immediately prior to operation, showing excess facial hair. **B:** Patient in 1950, two years after successful surgical removal of adrenocortical adenoma, showing persistence of some of the facial hair.

Upon examination of the infant, no abnormalities were found and he has developed normally to this writing.

It is not surprising that so few cases of pregnancy following surgical removal of masculinizing adrenocortical tumors are reported. Since these tumors are quite uncommon, there are very few cases in the age group in which pregnancy may be anticipated. Many adrenocortical tumors are malignant, and thus there is often a fatal outcome despite surgery; moreover some patients die as a result of the surgical procedure, although at present there is less risk than formerly. Since some of the masculinizing changes induced by these tumors are not reversible, marriage of these masculinized girls is unlikely, thus further reducing the chances of pregnancy.

It is of interest that pregnancies resulting in normal offspring have been also reported after the successful surgical removal of masculinizing tumors of the ovary (5). It is evident that masculinization due to adrenocortical or ovarian tumors does not preclude the possibility of normal pregnancy, if the tumor be successfully removed. Whether this is also true if the tumor

has been present and actively secreting masculinizing hormones for many years, is not clear.

In view of the fact that women masculinized as a result of congenital adrenal hyperplasia can now be treated with cortisone so that further masculinization is arrested and feminization with the establishment of normal menstrual periods results, it will be of interest to note any pregnancies that may occur in this group of patients.

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DECREASED BLOOD UREA LEVELS AFTER RESECTION OF ADRENOCORTICAL TUMOR FOR CUSHING'S SYNDROME

TO THE EDITOR:

The concentration of plasma or serum urea nitrogen ordinarily increases after a major operation. A simultaneous increase in the rate of excretion of urea shows this to be due to increased urea production from endogenous sources (sufficient to overcome the effect of decreased protein intake) rather than to a decreased renal clearance of urea.

Some years ago, during studies on the endocrine regulation of urate metabolism, it was noted that, following successful resection of hyperfunctioning adrenal adenomata in 2 patients with Cushing's syndrome, there occurred a striking postoperative fall in plasma urea concentration as well as the expected rise in urate concentration (Table 1). Because serum creatinine concentrations had not been determined the remote possibility could not be ruled out that the fall in urea concentration resulted from vascular readjustments and increased glomerular filtration rate rather than from increased protein anabolism.

TABLE 1. RISE IN PLASMA URATE LEVEL AND FALL IN PLASMA UREA LEVEL AFTER SUCCESSFUL RESECTION OF HYPERFUNCTIONING ADENOMATA IN PATIENTS WITH CUSHING'S SYNDROME

(These data are adapted from Wolfson *et al.: J. Clin. Endocrinol.* **9**: 760, 1949. All postoperative samples were taken in the first two postoperative weeks. Both patients were clinically cured by operation.)

No. of patients	No. of samples	Plasma total urate (mg./100 ml.)	Plasma urea nitrogen (mg./100 ml.)	Remarks
2	4	3.89	16.9	Preoperative
2	3	5.93	4.0	Postoperative

Recently, we have had the opportunity to observe the effect of a similar resection in a patient with Cushing's syndrome due to adrenocortical carcinoma. Although her operation temporarily greatly decreased steroid production, as shown in Table 2, there was eventual recurrence of the disease due to metastases. However, the postoperative remission was sufficiently long so that the simultaneous behavior of blood urea nitrogen and serum creatinine concentrations could be observed. Figure 1 summarizes the findings and shows that the typical fall in the circulating urea nitrogen level was not accompanied by proportionate change in serum creatinine concentration.

TABLE 2. PREOPERATIVE AND POSTOPERATIVE URINARY STEROIDS IN CUSHING'S SYNDROME DUE TO ADRENOCORTICAL CARCINOMA

(*See also top of next page*)

(Postoperative samples were taken between the tenth and thirtieth postoperative days. Except when otherwise explicitly stated, urine data are expressed in milligrams per gram of urinary creatinine per day. By means of an unpublished method in which Pettenkofer chromogen color is developed at room temperature, the total Pettenkofer chromogen may be differentiated into 1) the "estimated maximum dehydroepiandrosterone" fraction which develops its color at the same rate as the standard dehydroepiandrosterone, and 2) the "rapidly chromogenic Pettenkofer chromogen," (chiefly corticosterone metabolites) which develops color much more rapidly at room temperature.

The continued high corticoid excretion after operation clearly implies the existence of functioning metastases. The estimated rates of corticoid secretion are based on recovery experiments in which normal adults received large amounts of the steroids by mouth. The findings, therefore, should be read as follows: From previous recovery studies, a normal adult given 620 mg. of hydrocortisone and 130 mg. of corticosterone by mouth divided into four doses per day would be expected to show a urinary steroid pattern similar to that shown by this patient before operation.)

TABLE 2—*Continued*

	Preoperative	Postoperative	Difference
Butanol-extractable blue tetrazolium-reducing corticoid:	179 [3]	44 [1]	−135
Butanol-extractable Porter-Silber reactive corticoid:	145 [3]	34 [1]	−111
1.25 (17-Ketosteroid produced by sodium bismuthate):	153 [1]	85 [1]	− 68
Preformed 17-ketosteroid:	127 [1]	40 [2]	− 87
Total Pettenkofer chromogen:	33 [1]	13 [2]	− 20
Rapidly chromogenic Pettenkofer chromogen:	6 [1]	5 [2]	− 1
Normally chromogenic Pettenkofer chromogen:	27 [1]	8 [1]	− 19
Analysis of urinary steroid pattern			
17-Hydroxy-20-keto-21-hydroxycorticoid:	145	34	−111
17-Hydroxy-20-hydroxy-21-hydroxycorticoid *plus* 17-hydroxy-20-hydroxy-21-desoxycorticoid:	8	49	+ 41
Total 17-hydroxy-corticoid derivatives:	311	151	−160
17-desoxy-20-keto-21-hydroxy-corticoid:	34	10	− 24
Total 17-desoxy-corticoid derivatives:	42	16	− 26
Total urinary derivatives of corticoids:	353	167	−186
Estimated maximum dehydroepiandrosterone:	27	8	− 19
Pettenkofer-17-ketosteroid ratio:	0.26	0.33	0.23
Maximum DHA/17-ketosteroid ratio:	0.21	0.20	0.22
Estimated rates of corticoid secretion			
Estimated hydrocortisone secretion, mg. per day:	620	212	−408
Estimated corticosterone secretion, mg. per day:	130	38	− 92
Estimated hydrocortisone/corticosterone ratio:	4.8	5.6	4.4
Excretion of creatine and creatinine			
Urinary creatine:	958 [1]	305 [2]	−653
Creatinine coefficient, mg./Kg./day:	14.8	10.7	4.1

It seems likely that this disproportionate fall in plasma and blood urea concentration represents an index of the marked protein anabolic activity which occurs upon relief of the extreme hyperglycocorticoid state of Cushing's syndrome. The abrupt withdrawal of the great excess of corticoids unmasks a condition of protein depletion or "hungry cells" akin to the "hungry bones" seen when a hyperfunctioning parathyroid adenoma is removed from a patient with osteomalacia due to hyperparathyroidism.

The need for a biochemical index of protein anabolism which is simpler than nitrogen balance is widely appreciated. There is no reason to believe that the fall in the level of plasma urea relative to that of creatinine occurs only in the protein-anabolic state produced by the termination of hypercorticism. On the contrary, its long-known regular occurrence during normal pregnancy suggests that it can take place during rapid protein anabolism, even though adrenal function is normal or above normal. Experiments

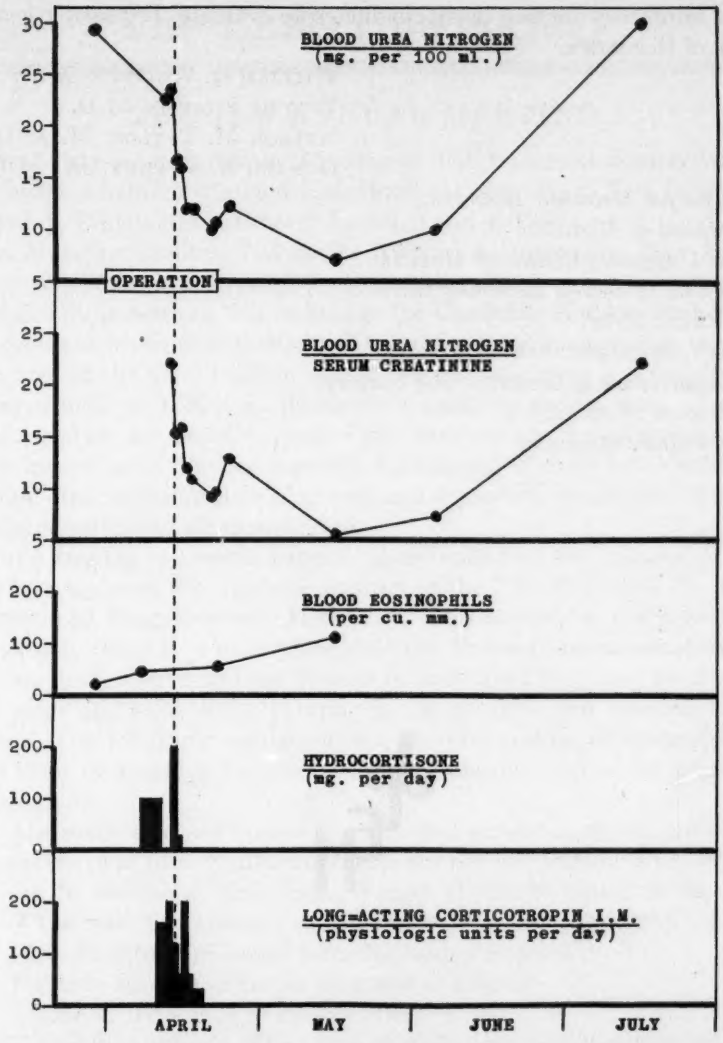

FIG. 1. *Fall in level of blood urea nitrogen (absolute and relative) compared to that of serum creatinine, following resection of an adrenocortical carcinoma in a patient with Cushing's syndrome.* (The postoperative course was uneventful and the patient was discharged on the seventh postoperative day. Ascites due to metastases appeared ir the latter half of June. Note that: 1) the sizable amounts of hydrocortisone and corticotropin given to stimulate the atrophic contralateral adrenal failed to prevent the fall in the blood urea nitrogen level, and 2) this fall persisted long after the patient had resumed normal food intake.)

in this latoratory are now being conducted to evaluate the possible general utility of this index.

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The Endocrine Society

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

Those wishing to present papers, (time limited to ten minutes) should send four copies of the title and abstract to the Vice-President, Dr. E. B. Astwood, 30 Bennet Street, Boston 11, Massachusetts, not later than *February 1, 1955*. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. Neither footnotes nor acknowledgments to sponsors can be published. References, if used, should be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading should be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institute of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, as there will be no opportunity for proof reading by authors.
5. Abstracts not conforming to the foregoing regulations will be returned to the authors.

THE 1955 AWARDS AND FELLOWSHIPS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be

divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1955 ANNUAL MEETING

The next annual meeting of the American Goiter Association will be held in Oklahoma City, Oklahoma, at the Skirvin Hotel, April 28, 29 and 30, 1955.

VAN METER PRIZE AWARD FOR 1955

The American Goiter Association again offers the Van Meter Prize Award of Three Hundred Dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in Oklahoma City, Oklahoma, April 28, 29 and 30, 1955, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed three thousand words in length, must be presented in English, and a typewritten double-space copy in duplicate sent to the Secretary, John C. McClintock, M.D., 149½ Washington Avenue, Albany, New York, *not later than January 15, 1955.* The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the Annual Transactions of the Association.

AMERICAN GOITER ASSOCIATION AWARD OF MERIT FOR 1955

The American Goiter Association Award of Merit and its honorarium of $1,000.00 is again offered by its anonymous donor for 1955. Brown Dobyns, M.D., received this award in 1954.

The terms of the gift specify that this sum shall be given to a member of the Association, under 45 years of age, who has made the most important contributions to the knowledge and understanding of thyroid disease or the treatment of thyroid disease.

Candidates for this award are to be nominated by members of the Association. When making a nomination complete details of the nominee's present and past positions and training must be submitted. A complete

bibliography of the nominee's publications is required. A full explanation of the reasons for nominating the candidate must be given.

The candidates submitted are to be reviewed by an anonymous committee appointed by the president. The chairman of this committee will present the deliberations and decisions of said committee to the Executive Council in advance of the annual meeting.

The award is to be called The American Goiter Association Award of Merit and it shall be presented at the annual banquet.

Members of the American Goiter Association are requested to send nominations in duplicate to this secretary, making certain the information required by the deed of gift is included. All nominations must be received *not later than February 1, 1955.*

JOHN C. MCCLINTOCK, M.D.
Secretary,
149½ Washington Ave.,
Albany 10, N. Y.

BOOKS RECEIVED

Color Atlas of Pathology. Volume II. Prepared under the auspices of the U. S. Naval Medical School of the National Naval Medical Center, Bethesda, Maryland. Endocrine System, Including Pituitary, Thyroid, Parathyroid, Adrenals and Pancreas—Gynecology and Obstetrics, Including Reproductive Organs—Breasts—Male Genital Tract—Skin; 460 pages; illustrated with 1,032 figures in color on 343 plates; 1954. Philadelphia, J. B. Lippincott Company. Price $20.00.

Current Concepts in Digitalis Therapy. By BERNARD LOWN, M.D. and SAMUEL A. LEVINE, M.D., Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.; 174 pages; 10 tables; 21 figures; extensive bibliography; 1954. Boston, Little, Brown and Company. Price $3.50.

(The) Female Sex Hormones. By LEON J. DEMERRE, Ph.D.; 225 pages; 1954. NewYork, Vantage Press, Inc. Price $3.50.

Medizin, Chemie und Krebs. I. Die Geschlechtlichkeiten und ihre Auswirkungen. Edition 2. By DR. HANS TRUTTWIN, Dozent, Staatliches Institut für Volksgesundheit, Stockholm; 166 pages; 70 diagrams; extensive bibliography; 1954. Vienna, Austria, Wilhelm Maudrich, Publisher. Gln.geb. DM 27.

The Pineal Gland. A Review of the Physiologic Literature. By JULIAN I. KITAY, M.D. and MARK D. ALTSCHULE, M.D., Harvard Medical School and Beth Israel Hospital, Boston, Mass.; McLean Hospital, Waverly, Mass.; 294 pages; 78 tables; 3 figures; 1 color plate; bibliography of 1,248 items; 1954. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Mass. Price $5.00.

Principles of Internal Medicine. Edition 2. Edited by T. R. HARRISON, M.D., RAYMOND D. ADAMS, M.D., PAUL B. BEESON, M.D., WILLIAM H. RESNIK, M.D., GEORGE W. THORN, M.D. and M. M. WINTROBE, M.D. In ten parts: The Physician and the Patient, Cardinal Manifestations of Disease, Biologic Considerations, Reactions to Stress and to Antigenic Substances, General Care of the Patient, Metabolic and Endocrine Disorders, Disorders Due to Chemical and Physical Agents, Diseases Due to Biologic Agents, Diseases Associated with Reactions to Stress and to Antigenic Substances, Diseases of Organ Systems; 1,812 pages; 207 figures; 5 color plates; 149 tables; 1954. New York, The Blakiston Company, Inc. Student 1-volume edition $16.00. Professional 2-volume edition, boxed, $21.00.

Reproductive System. A Compilation of Paintings on the Normal and Pathologic Anatomy of the Reproductive System. Volume 2 of The Ciba Collection of Medical Illustrations. Prepared by FRANK H. NETTER, M.D.; edited by ERNST OPPENHEIMER, M.D.; foreword by JOHN ROCK, M.D. Of the 233 plates (in color), 89 appear in print for the first time in this book. In fourteen sections: Development of the Genital Tracts and Functional Relationships of the Gonads, Normal Anatomy of the Male Genital Tract, Diseases of the Penis and Urethra, Diseases of the Prostate and Seminal Tract, Diseases of the Scrotum and Testis, Normal Anatomy of the Female Genital Tract and Its Functional Relationships, Diseases of the Vulva, Diseases of the Vagina, Diseases of the Uterus, Diseases of the Fallopian Tubes, Diseases of the Ovary, Pregnancy and Its Diseases, Anatomy and Pathology of the Mammary Gland, Intersexes; 302 pages; 233 colored plates; 1954. Commissioned and published by CIBA, Ciba Pharmaceutical Products, Inc., Summit, N. J. Bookstores are permitted to stock this volume with the understanding that it will not be sold at a price greater than that announced by Ciba—$13.00.